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# EFFECT OF REFINED HYDROGENATED KARANJA OIL ON LIPID METABOLISM IN ADULT MALE ALBINO RATS

## A COMPARATIVE STUDY

B. MANDAL[a], S. GHOSH MAJUMDAR[a] and C. R. MAITY[b, c]

[a] Department of Chemistry, University of Burdwan, Burdwan-713104, West Bengal. India

[b] Department of Biochemistry, Burdwan Medical College, Burdwan-713104, West Bengal. India

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A non-edible oil, karanja (*Pongamia glabra*) oil was extracted, refined by removing the toxic materials, colour, odour and bitterness and then it was hydrogenated. The refined hydrogenated karanja oil was supplemented to the diet of adult male albino rats at the extent of 15 percent for 30 days and the effects of the supplemented diet on plasma and liver lipids and the fatty acid composition of plasma and depot fat were studied. Coconut oil and corn oil were also used in separate diets at the same extent and period for comparison. Total lipid and cholesterol contents of liver and serum altered significantly with the nature of fat ingredients used in the experimental diets. Fatty acid composition of plasma was significantly influenced by the dietary fats and the changes reflected the dietary fat composition but the response of depot fat to the diet modification was slower than with plasma. It is obvious from the present study that the refined hydrogenated karanja oil (RHKO) could be incorporated in foods as a fat ingredient.

**Keywords:** gas liquid chromatography, karanja oil, dietary fat, fat ingredient

Karanja *(Pongamia glabra)* grows widely in India and is unique among higher plants for its oil seed which yields about 27 percent of a yellowish brown oil. Out of 1 110 000 tons of karanja seeds available in India annually only 25 900 tons are used i.e. at present hardly 23% of the total potential is being tapped (SWAMINATHAN, 1979). The oil is bitter in taste and has very unpleasant odour, becomes non-edible due to the presence of two toxic flavonoids, karanjin and pongamol. This non-edible fatty oil is now partially used for leather tanning and soap making after hydrogenation. It is also used in a variety of medicines, besides being anthelmintic, is reported to have a curative value for diseases of eyes, rheumatism, leucoderma and itching wounds (KIRTIKAR & BASU, 1918; COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, 1969). This oil has been found to possess synergistic activity for pyrethroids (PARMAR & GULATI, 1969; ATTRI et al., 1973; PARMAR et al., 1975). SUDBOROUGH and co-workers (1922) refined karanja oil (KO) by deacidification with aqueous caustic soda followed by bleaching with 3.5% Fuller's earth and compositional fatty acids were

[c] Request for reprints should be sent to

refined by GUPTA and MITRA (1953). Beside all these, inactivating the toxins by processing KO to upgrade it as an edible oil was overlooked which makes wastage of huge quantities of that oil seed.

The shortage and high prices of edible oil has stimulated nutritional and economic feasibility studies of different waste forest oil seeds as a good source of vegetable oil. It appears therefore that these wasted oil seeds need to be further exploited and thoroughly evaluated nutritionally. Studies with muhua *(Maduca indica)* have shown that refined and suitably processed fat can be used in confectionary and chocolate preparation (MULKI & GANDHI, 1977; COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH, 1962). GANDHI and co-workers (1976) showed that sal *(Shorea robusta)* fat could be safely used for edible purposes. After refining, the nahor *(Mesua ferrea)* oil is used as an edible oil (GUPTA, 1951; KAPADIA & AGGARWAL, 1954), and other studies on non-edible oil seeds are in progress.

In the present study raw KO was refined by removing its colour, bad odour and toxic constituents and then it was hydrogenated to upgrade it as an edible oil. The biological tests of refined hydrogenated karanja oil (RHKO) reveal its status regarding its edibility. With this end in view RHKO was nutritionally evaluated following well-established rat bioassay procedure. The biological indices measured were total lipids, phospholipids, cholesterol and free fatty acid (FFA) of serum and liver and the fatty acid composition of serum and depot fat. Food intake, growth performance and some blood biochemical parameters of rats were also measured.

## 1. Materials and methods

### *1.1. Preparation of RHKO*

Deep brown coloured KO was extracted from the ground karanja seed by Soxhlet solvent (petroleum ether, 60–80 °C) extraction method. The oil was kept in the refrigerator (0–4 °C) for 3 days and after that period a brownish-white semisolid mass had sedimented at the bottom of the container which was removed carefully. The remaining brown coloured oil was first steam distilled and then refined by alcohol, deacidification with aqueous caustic soda followed by bleaching with 3.5% Fuller's earth according to the method of SUDBOROUGH and co-workers (1922). The refined KO was then hydrogenated. Hydrogenation was carried out in a three-necked round-bottom glass flask at atmospheric pressure using rufort nickel catalyst (0.75% catalyst on the weight of oil; in the preparation of the catalyst 25% reduced nickel was employed). The oil was hydrogenated for the period of 5 h at 190 °C and the hydrogenated product, a white solid mass (m.p. 42 °C) was found to be devoid of bitterness and unpleasant odour. The product RHKO, was also free from the toxic pongamol

and karanjin, examined by following the usual tests (RANGASWAMI & SESHADRI, 1942; BHEEMASANKARA & VENKATSWARLU, 1956; JATKAR & MATTOO, 1954).

*1.2. Test animals and their diets*

Forty eight male albino rats of inbred strain (bred in our laboratory) each weighing approximately 150 g at the start of the experiment, were distributed into four groups of twelve animals, each. All the animals were caged individually and maintained under controlled temperature (21 ±1 °C) and humidity (55 %) conditions. Twelve animals in each of the four groups received the laboratory stock diet, the composition of which was (g per kg diet): casein 200, starch 400, cellulose powder 50, salt mixture 40, vitamin mixture 10 and sucrose to 1 kg. Salt mixture and vitamin mixture were added according to US PHARMACOPOEIA (1965) and NATIONAL RESEARCH COUNCIL (1963), respectively. Fats or mixture of fatty acids were added at the expense of sucrose. In the three experimental groups different kinds of dietary fats were used (g $kg^{-1}$): CN coconut oil 150, CO corn oil 150, RHKO 150. In the control group (N) a mixture of fatty acids (g per kg diet) was given in the diet: lauric acid 2.4, myristic acid 2.1, palmitic acid 15.5, stearic acid 2.4, oleic acid 12.4, linoleic acid 20.2 and arachidic acid 2.5. Rats were given the experimental diet and water ad libitum for 30 days, food intake and body weights were recorded daily and at the end of 30 days rats of all groups were sacrificed under similar conditions.

*1.3. Analytical methods*

Specimen portions of RHKO, coconut oil and corn oil used in the diets were subjected to fatty acid analysis by gas liquid chromatography following the method described later and the results were listed in Table 1.

*1.3.1. Blood analysis.* Blood haemoglobin (Hb) was measured by using a haemoglobinometer (Coulter Electronics, Hialeah, Florida, USA). Blood glucose and blood urea were estimated according to the methods of COOPER and DANILE (1970) and NETELSON (1957).

*1.3.2. Lipid analysis.* Extractions of serum and liver lipids were carried out according to FOLCH and co-workers (1957) using chloroform–methanol mixture and analysed for cholesterol (SPERRY & WEBB, 1950), phospholipid (FISKE & SUBBAROW, 1925), FFA (HEINDEL et al., 1974) and total lipid was estimated by evaporating the measured amount of extract.

*1.3.3. Fatty acid analysis.* For fatty acid analysis, 100 mg depot fat and 0.2 $cm^3$ plasma were used and lipid materials were extracted by the method of FOLCH and co-workers (1957). The lipid extract was washed with 0.1 *M* potassium chloride and evaporated to dryness. The dry mass was saponified at

110 °C for 24 h in 5 $cm^3$ ethanolic potassium hydroxide solution. Saponified fatty acids were further extracted with redistilled solvent ether, methylated with diazomethane and applied to a 200 g diethylene glycol succinate per kg chromosorb WHMDS column of a Perkin Elmer F 11 gas liquid chromatograph for fatty acid analysis.

*1.3.4. Statistical analysis.* Statistical analyses were done according to Student's *t* test.

## 2. Results

Fatty acid composition of the dietary fat ingredients are shown in Table 1 which shows that RHKO contained the highest percentage of fatty acid 18:1 compared to the two other fat ingredients whereas fatty acid 18:2 was comparatively high in corn oil. Coconut oil was rich in saturated fatty acids but lacked 18:1 and 18:2 fatty acids. Fatty acid composition of the fat ingredients suggested that RHKO resembles corn oil more in some respect than coconut oil.

Table 1

*Composition of fatty acids in coconut oil, corn oil and RHKO used in the diets*

| Fatty acids | Fatty acid content of oils (mg $g^{-1}$) | | |
|---|---|---|---|
| | Coconut | Corn | RHKO[a] |
| Below 12:0 | 194 | — | — |
| 12:0 | 472 | — | 10 |
| 14:0 | 164 | 17 | 18 |
| 16:0 | 85 | 110 | 98 |
| 16:1 | 11 | 16 | 12 |
| 18:0 | 17 | 29 | 187 |
| 18:1 | 55 | 488 | 530 |
| 18:2 | trace | 340 | 101 |
| 20:0 | 2 | — | 25 |
| 20:4 | — | — | — |
| Others | — | — | 9 |

[a] RHKO contains approximately 20 percent of trans-isomeric fatty acids
The data are mean values of two determinations

Table 2 summarizes the results obtained for food intake, weight gain and the concentration of blood Hb, sugar and urea. There were no statistically significant differences in food intake between the rats given different dietary fats in individual experiments. In general, weight gain was greater in the rats given fat at 150 g per kg diet as compared to that of controls, but the differences were not significant. Table 2 also reveals that concentrations of blood Hb and urea were not influenced by the nature of dietary fat. Slightly increased blood sugar level was observed in the rats on fat supplemented diet over controls.

Table 2

*Food intake, body weight gain, blood Hb, blood sugar and blood urea levels in rats given diets supplemented with some fats or a normal diet*

| Diet | Food intake (g per day) | | Body weight gain (g) | | Haemoglobin level (g $dm^{-3}$) | | Blood sugar (mg $dm^{-3}$) | | Blood urea (mg $dm^{-3}$) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| N | 15.4 | 1.1 | 75 | 5 | 152 | 7 | 842 | 22 | 352 | 12 |
| CN | 16.1 | 0.8 | 85 | 4 | 156 | 8 | 888 | 23 | 346 | 11 |
| CO | 15.8 | 0.9 | 82 | 6 | 158 | 6 | 898 | 21 | 345 | 14 |
| RHKO | 16.2 | 0.7 | 85 | 5 | 154 | 9 | 894 | 19 | 348 | 16 |

$\bar{x}$: mean value calculated from six rats
$\pm s$: standard deviation
N: normal diet
CN: coconut oil
CO: corn oil
RHKO: refined hydrogenated karanja oil

The effects of different dietary fats on the concentration of plasma and liver lipids are given in Table 3. Total lipid content of plasma and liver significantly increased in all the groups as compared to that of control. Both the corn oil and RHKO showed hypocholesteremic effect. Cholesterol content of

Table 3

*Effects of some supplementary dietary oils (150 g $kg^{-1}$) fed for 30 days or of a normal diet on serum and liver lipid composition in albino rats*

| | Units | N | | CN | | CO | | RHKO | |
|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| Plasma: | | | | | | | | | |
| Total lipid | (mg $dm^{-3}$) | 2780 | 60 | 3212*** | 74 | 3040 | 52** | 3054 | 72* |
| Phospholipid | (mg $dm^{-3}$) | 856 | 18 | 884 | 15 | 832 | 15 | 842 | 22 |
| Cholesterol | (mg $dm^{-3}$) | 684 | 18 | 786 | 141 | 588 | 25** | 612 | 24* |
| Free fatty acid | (m$M$ $dm^{-3}$) | 0.32 | 0.04 | 0.44* | 0.03 | 0.45 | 0.05* | 0.43 | 0.03* |
| Liver: | | | | | | | | | |
| Total lipid | (mg $dm^{-3}$) | 125 | 4 | 142* | 5 | 138* | 4 | 132 | 3 |
| Phospholipid | (mg $dm^{-3}$) | 78 | 2 | 82 | 4 | 74 | 3 | 76 | 6 |
| Cholesterol | (mg $dm^{-3}$) | 6.2 | 0.4 | 7.2 | 0.6 | 5.1 | 0.7 | 5.4 | 0.8 |
| Free fatty acid | (mg $dm^{-3}$) | 3.4 | 0.7 | 3.8 | 0.5 | 3.6 | 0.4 | 3.7 | 0.4 |

* Significant at $P \leq 0.05$ probability level
** Highly significant at $P \leq 0.01$ probability level
*** Very highly significant at $P \leq 0.001$ probability level
For diets and symbols see Table 2

Table 4

*Composition of total fatty acids of plasma from rats fed different oil-supplemented diets or a normal diet*

| Diets | Fatty acids of plasma (mg $g^{-1}$) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12:0 | | 14:0 | | 16:0 | | 16:1 | | 18:0 | | 18:1 | | 18:2 | | 20:4 | | Others | |
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| N | 18 | 5 | 30 | 4 | 212 | 12 | 54 | 8 | 90 | 8 | 208 | 8 | 248 | 6 | 88 | 9 | 52 | 10 |
| CN | 71*** | 11 | 52** | 5 | 208 | 15 | 56 | 8 | 85 | 12 | 181* | 9 | 222* | 8 | 72 | 12 | 46 | 5 |
| CO | 10 | 4 | 20 | 5 | 201 | 13 | 50 | 6 | 78 | 11 | 231* | 6 | 301*** | 9 | 74 | 14 | 35 | 12 |
| RHKO | 12 | 4 | 19 | 5 | 198 | 18 | 51 | 7 | 88 | 13 | 268*** | 8 | 237 | 12 | 82 | 8 | 45 | 9 |

For legend and symbols see Tables 2 and 3

liver also changed in a similar pattern but with less intensity. Phospholipid content of serum and liver increased with coconut oil supplemented diet and decreased with RHKO and corn oil but the differences never are significant. In general FFA level of plasma was significantly greater in rats given fat-rich diets compared to controls but liver FFA level remained unaltered or slightly modified.

The fatty acid composition of plasma from rats maintained on diets supplemented with different fats and those for rats on a normal diet are shown in Table 4. In rats which were fed on a normal diet, the level of fatty acids 12:0 and 14:0 were 18 and 30 mg $g^{-1}$, whereas in the animals given coconut oil supplemented diet the levels were 72 and 52 mg $g^{-1}$, respectively, which are significantly higher ($P \leq 0.001$ and $P \leq 0.01$). In rats fed on corn oil and RHKO supplemented diet the levels were found to be lower but the changes were not significant. The level of fatty acid 18:1 was reduced significantly ($P \leq 0.05$) in rats on coconut oil diet while increased significantly in those on corn oil ($P \leq 0.05$) and RHKO ($P \leq 0.05$) diet as compared to that of controls. The level of fatty acid 18:2 changed also in a similar pattern. It was significantly ($P \leq 0.05$) reduced to 222 mg $g^{-1}$ total fatty acid in rats on coconut oil supplemented diet from the control value of 248 mg $g^{-1}$ while in rats on corn oil supplemented diet it was raised significantly ($P \leq 0.001$) to 301 mg $g^{-1}$. The level of fatty acid 18:2 in rats with RHKO supplemented diet was 237 mg $g^{-1}$ which was lower than the control value but this change was non-significant. The levels of fatty acids 16:0, 16:1, 18:0 and 20:4 were found to be almost similar in the test animals on normal diet and diets supplemented by different fats although they were slightly modified by the fatty acid composition of the diet.

The response of fatty acid composition of the depot fat (Table 5) to 30 days of diet modification was much lower than that of plasma, though the

Table 5

*Composition of total fatty acids of depot fat from rats fed different oil-supplemented diets or a normal diet*

| Diets | Fatty acids of depot fat (mg $g^{-1}$) | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12:0 | | 14.0 | | 16:0 | | 16:1 | | 18:0 | | 18:1 | | 18:2 | | 20:4 | | Others | |
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| N | 30 | 4 | 50 | 5 | 208 | 12 | 75 | 9 | 68 | 8 | 352 | 12 | 141 | 5 | 38 | 5 | 38 | 6 |
| CN | 72** | 12 | 85* | 12 | 205 | 15 | 68 | 9 | 63 | 7 | 320* | 7 | 114*** | 3 | 34 | 6 | 37 | 3 |
| CO | 24 | 6 | 45 | 6 | 205 | 18 | 67 | 7 | 61 | 8 | 389* | 6 | 150 | 7 | 34 | 4 | 34 | 7 |
| RHKO | 28 | 3 | 51 | 9 | 201 | 21 | 66 | 11 | 71 | 4 | 388* | 11 | 132 | 6 | 30 | 7 | 33 | 8 |

For legend and symbols see Tables 2 and 3

change reflected the dietary fatty acid composition. Coconut oil supplemented diet increased the level of fatty acids 12:0 and 14:0 and reduced those of 18:1 and 18:2 significantly, while both, corn oil and RHKO supplemented diets raised the fatty acid 18:1 level significantly ($P \leq 0.05$) as compared to that of control. Other fatty acid levels remained unaltered or slightly modified by the diet but the changes never reached statistical significance.

## 3. Conclusions

The fatty acid profile of RHKO appear to fall within the range of values seen in edible oils and contains high amounts of unsaturated fatty acids and a low amount of saturated fats even after hydrogenation. It seems probable that the extent of hydrogenation is low. KANE and KULKARNI (1954) showed that karanja oil could be hydrogenated only to the extent of 15% and even after hydrogenation the amount of unsaturated fatty acids exceeded that of saturated fats. There were no toxic oxygenated fatty acids such as epoxy, keto or hydroxy acids as revealed by gas liquid chromatography (GLC). It was also found that the unsaturated fatty acid components of RHKO became partly isomerised during hydrogenation and the extent of trans-isomerisation was approximately 20 percent as determined by capillary column GLC as described by JAEGER and co-workers (1975). Thus the presence of these trans-isomers of unsaturated fatty acids in the RHKO caused a deficiency in the content of essential fatty acids. It was reported by FUNCH and co-workers (1960) and KOHN and POKORNY (1964) that trans fatty acids were deposited in the depot fat almost in the same concentration as found in food and probably metabolised like other fatty acids without any deleterious effect. RHKO used in the present study showed no adverse nutritional effects which supports the earlier reports.

Diets supplemented with different fats slightly improved the growth response which could be attributed to the high fat content of the diet and its utilization and metabolism. Hb or urea level of the blood did not show any noticeable change, (these are known as acceptable common parameters to assess the apparent ill effect if any).

Table 3 shows that the lipid composition of the serum and liver were influenced by the nature of dietary exogenous fat. Serum and liver total lipid levels were found to be significantly high in all the animals on fat supplemented diets over controls and this may be due to high exogenous fat intake and its utilisation in the body. Regarding the nature of dieteary fat it was found that coconut oil in the diet elevated the cholesterol level of serum and liver while the corn oil and RHKO caused a significant decrease in the cholesterol level of both the serum and liver. It was shown by KINSELL and co-workers (1953) that lack of unsaturated fatty acids in the diet may result in the elevation of serum cholesterol whereas reduction in serum cholesterol seems to be directly related to the excess amount of unsaturated fatty acids in the diet (GOPALAN et al., 1960). The real mechanism by which poly-unsaturated or unsaturated oils in the diet decrease plasma cholesterol is unknown. BYERS and FRIEDMAN (1958) and GORDON and co-workers (1957) suggested that unsaturation promotes the catabolism of cholesterol, causing its excretion in the form of fecal bile acids. Thus the cholesterol lowering action of RHKO and corn oil may be explained by their degree of unsaturation. In this context it should be pointed out that RHKO contains some trans isomeric fatty acids and is poor in essential fatty acids. It was found that a dietary fat highly unsaturated but poor in essential fatty acids reduced the concentration of serum cholesterol (AURENS et al., 1959).

Except cholesterol, FFA levels of serum were significantly affected by the fats supplemented in the diets and it was pointed out by ALBRINK and co-workers (1958) that the only significant change in the serum lipids following a fatty meal occurs in the triglycerides and FFA fraction. DOLE (1956) has also reported that the concentration of FFA in plasma of human beings is dependent on the nutritional state, increases during fasting and decreases after high carbohydrate intake. LAURELL (1956) found a high concentration of plasma FFA during starvation of normal adults. GORDON and CHERKES (1956) were the first to postulate an important role of FFA fraction as transport form of fat from depot fat to different tissues for oxidation. FFA is offered to tissues as the alternative fuel to glucose and its metabolic oxidation is vital in starvation or low carbohydrate high fat diet. At high FFA concentration, as seen in starvation, the rapid rate of disappearance of plasma fraction FFA (up to 0.5 MEQ per $dm^3$ $min^{-1}$) caused an energy production of around 20.934 kJ per day in an adult human, assuming that all the FFA leaving the circulation is directly oxidised (FREDRICKSON & GORDON, 1958). The outflow of FFA from the

fat depots is dependent on the state of carbohydrate metabolism but the mechanism by which the FFA outflow from the depots is regulated is unknown, except for the fact that all the factors which decrease the rate of glucose utilisation increase the outflow of FFA (GORDON & CHERKES, 1956; BIERMAN et al., 1957). In the present study high fat diet increased plasma FFA level significantly which may be due to increased lypolysis of depot fat following high fat diet. In this regard, it seems probable that carbohydrate metabolism was depressed due to increased FFA level. Depressed carbohydrate utilisation in the rats on fat diet may be deduced from the blood glucose levels which were slightly higher than the control value as shown in Table 2 and it is probable that lower glucose utilisation increased the blood glucose level slightly.

It is now well established that the fatty acid composition of depot fat is influenced by dietary fat (ANDERSON & TOWE, 1964; BRAUN et al., 1967). It is also appreciated that the response of depot fat to diet modification is substantially delayed, in contrast to changes in blood lipids (MAJIMA & CURATA, 1967). It was pointed out by SHEPHERD and co-workers (1980) that although the diet-induced change in fatty acids composition of depot fat is slow, measurable effects become increasingly apparent after 14 days of treatment. In this study it was shown that the fatty acid composition of depot fat reflects dietary fatty acid significantly after 30 days of intake. Although fatty acid composition of depot fat reflects dietary fat, it is shown in Table 5 that saturated oils influenced this parameter to a greater extent than the unsaturated oils used in the present study.

In the light of the present results it appears therefore that RHKO causes no growth retardation, health hazards or toxic effects on the biochemical parameters of rats. Thus, RHKO could be incorporated in foods as a fat ingredient.

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# HEAT CONSERVATION OF SOFT DRINKS PREPARED WITH ENZYMES

E. SZILÁGYI-TÓTH[a], O. REICHART[b] and K. ZETELAKI-HORVÁTH[a]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] University of Horticulture, H-1118 Budapest, Somlói út 16. Hungary

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A heat preservation method of soft drinks, produced by enzyme technology, was elaborated.

As a model, fibrous carrot juice was used, and parameters of thermal death have been determined on a mixed flora with the help of a new dynamic method. The new method enabled to obtain the individual thermal death curves as a result of one measuring series, eliminating thereby various sources of error of the traditional methods. On the basis of the survival and death curves, the important parameters of thermal death ($D$ and $z$, resp.) have been determined.

The pH dependence of the values of $D$ and $z$ were tested in the range of pH 3 to 7. The decimal reduction time ($D$) proved to be pH dependent between pH values of 3 and 5.5. The pH and the $z$ values did not show a close correlation.

According to the analysis of covariance the slope of the death curves belonging to various pH values did not differ significantly from each other. In consequence the thermal death for the soft drinks can be characterized by a common $z$ value.

According to the results of these experiments, a flash pasteurization at 80 °C for 0.5 min was suggested.

During the six months storage, the microbiological state of the pasteurized samples proved to be of high grade, which was supported by the biological values as well as by the organoleptic properties of the drinks.

**Keywords:** carrot juice, thermal death, enzymic technology, heat treatment

The production of fibrous soft drinks (vegetable and fruit cocktails) by enzyme technology has been developed at the Bioengineering Department of the Central Food Research Institute. The aim of this work was to elaborate a method of heat treatment by the use of which a good microbiological stability of the products could be achieved with the least negative alteration of the valuable components (nutrients, vitamins and flavour) of the original material.

Fibrous soft drinks prepared from vegetables and fruits possess the properties of the original basic materials, e.g. colour, flavour, etc. and contain almost the whole nutrient, vitamin and mineral content of the original tissues. (ZETELAKI-HORVÁTH & GÁTAI, 1977; ZETELAKI-HORVÁTH & VAS, 1980). By the use of endo-polygalacturonase, which degrades the middle lamella between the cells of the plant (vegetable and fruit) tissues, the processing loss (straining or pressing loss) can be decreased by 10–30% when compared to those processed without enzyme treatment.

Considering the new product and the new technology, it was necessary to investigate all parameters of heat treatment influencing the heat destruction of microbes, enabling to determine the optimal conditions of pasteurization.

## 1. Materials and methods

### *1.1. Preparation of soft drinks*

As model materials carrot nectar (one of the most contaminated components of some cocktails), and a carrot-apple (C-A) cocktail were used for our investigation.

Washed and steamed (80 °C for 15 minutes) carrots (of unidentified variety) were disintegrated by a household vegetable grinder.

Liquid endo-polygalacturonase was added to the carrot in a concentration of 0.5% (v/w). Enzyme treatment was carried out in a buffer of pH 3.8 at a liquid–vegetable ratio of 1:1, and at a temperature of 40 °C. Continuous mixing was ensured on a shaking machine of 330 r.p.m. for 1 hour.

After incubation, samples were strained through a 1 mm sieve of a household equipment (AKA Electric, GDR).

In the case of apples no steaming was used and the enzyme was added to the ground fruit without any buffer in a concentration of 0.05% (v/w).

### *1.2. Enzyme preparation*

A liquid enzyme preparation "Phylendonase", with a specific activity ($SPA_{75}^{Na-P}$, of 300 $cm^3\ h^{-1}\ cm^{-3}$ was used. The enzyme is produced by an *Aspergillus awamori* strain in the fermentation factory of Phylaxia (Budapest), according to a fermentation technology developed at the Bioengineering Department of the Central Food Research Institute (Zetelaki et al., 1981).

### *1.3. Microbiological investigation of soft drinks*

The number of survivals of the heat treated samples was determined by the agar plate count method.

The number of survivals was determined from different dilutions by calculating the logarithmic mean.

For the determination of the number of residual spore-forming microorganisms, samples were incubated at 80 °C for 10 minutes before plate counting.

### *1.4. Method of heat treatment*

Pasteurization of the test material was carried out in a laboratory experimental equipment constructed specially for this work (Fig. 1).

Test materials of a temperature of $T_0$ were heated continually in an ultrathermostat to reach the different temperatures ($T_1$) used for pasteurization. The holding time at every temperature was 0.5 min followed by a rapid cooling to temperature $T_2$.

The pasteurized fluid was filled in sterile 250 cm³ brown flasks, which were closed by crown-caps.

The temperatures of pasteurization (according to results of preliminary experiments) were for carrot juice: 338, 348, 358 and 368 K (65, 75, 85 and 95 °C); for C–A cocktail: 338, 348 and 358 K (65, 75 and 85 °C).

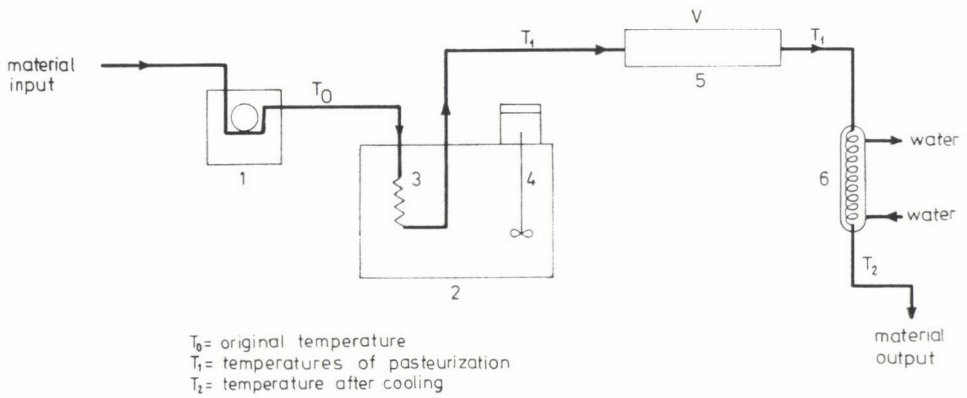



Fig. 1. Scheme of the laboratory pasteurizing equipment. 1: S 33 peristaltic pump ($W = 4\,cm^3s^{-1}$); 2: U-10 ultrathermostat; 3: heat exchanger (t = 30 s); 4: mixer; 5: holding section ($V = 120$ cm³); 6: cooler

The duration of heat treatment was the same (0.5 min) at every temperature used.

### *1.5. Death rate determination of mixed microbial flora*

The essence of the method is that by it becomes possible to construct the thermal death curve by a series of measurements in a single identical system (REICHART, 1979).

The temperature of the microbial suspension to be investigated is continuously increased and the viable cell counts determined point by point and the temperature are plotted against time. The death rate coefficients and the decimal reduction times belonging to different temperatures are determined from the difference quotients according to time of the anisothermal survival curve. Since neither the age of the culture, nor the heat treatment medium, nor the composition of the nutrient medium changes in the course of the experiment, the results reflect changes caused solely by changes in temperature.

*1.6. Storage experiments*

The microbiological state of the pasteurized samples (prepared according to the method given under section 1.4) was investigated as a function of storage period. One part of the samples was stored at room temperature (about 22–23 °C) and the rest in a refrigerator (at +4 °C). The cell count was determined monthly.

*1.7. Methods of evaluation*

Parameters of death rate of the microflora upon heat treatment of soft drinks were determined in two ways.

In the traditional isotherm method the 0 values were calculated from the regression equations of the linear section of the survival curves.

The results obtained by the new method were evaluated as follows: The temperature and the logarithm of the viable cell count were plotted against time then the best fitting curves were drawn. The values read from the curves served for calculation. The slope of the survival curve was calculated point by point and from this the death rate coefficient and the $D$ value were obtained. The temperature belonging to a given time was interpolated and the logarithm of the $D$ value was plotted as a function of this. Thus the points of the thermal destruction curve were obtained and the $z$ value was calculated by regression analysis.

Standard deviation of the number of survivals was less than 0.2 logarithmic unit which is not marked on the figures in order to avoid their complexity.

## 2. Results

*2.1. Results of microbiological investigations of the samples*

*2.1.1. Initial number of microbes.* Microbiological investigations were made from 7 carrot nectars and one carrot-apple cocktail. At first the survival numbers of mesophilic aerobic microorganisms, the number of moulds and yeasts and the survivors of spore-forming microorganisms (after 10 minute heat treatment at 80 °C) were determined.

The ratios of survivors were compared by analysis of variance. According to the results of analysis of variance, significant differences were found within the three groups at a 95% probability level (Fig. 2).

*2.2. Results of thermal destruction experiments*

*2.2.1. Changes of decimation time as function of pH.* Investigation of the thermal death rate was started with the determination of the pH dependence of decimation time.

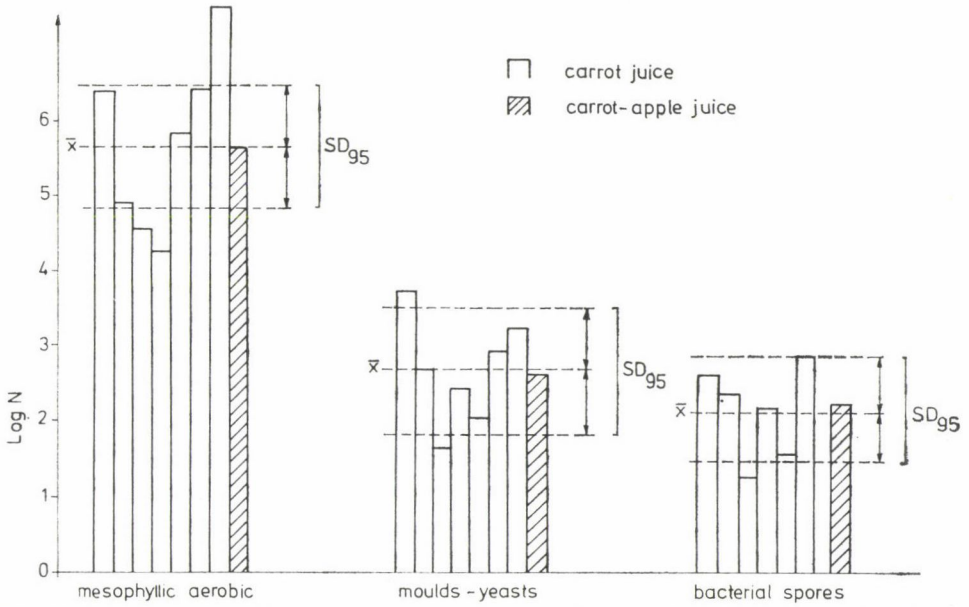



Fig. 2. Comparison of the number of survivors in the samples by analysis of variance

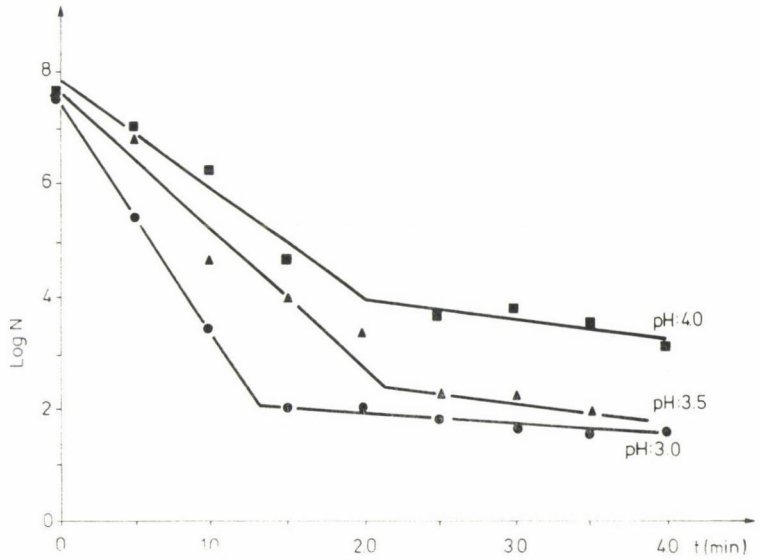



Fig. 3. Survival curves of the microflora of carrot juice as a function of the pH

From the samples (pH: 5.25) a series of different pH has been prepared (from pH 2 to 7). The isothermal measurements were carried out at a temperature of 60 °C with 0.5 minute sampling.

The obtained survival curves consisted of two sections at the nine pH values tested. The first, steeper section of the lines represent the rapid death

of the less resistant microflora, while the second section of the lines characterizes the death rate of the resistant microorganisms. The survival curves obtained at pH 3.0, 3.5 and 4.0, respectively, are shown in Fig. 3.

The nine $D$ values of the first sections varied between 0.3 and 0.7 minutes. In the pH range of 3.0–3.5, $D$ values increased at a constant rate, but above pH 5.5 the effect of pH decreased. The effect of pH on the heat destruction of the resistant part of the microflora is more significant than in the first section of the survival curves. This effect can be seen in Fig. 4.

*2.2.2. Changes of* z *values as a function of pH.* Value $z$ is a very important characteristic of the thermal death of microorganisms ($z$ = the temperature difference necessary to increase the thermal death rate tenfold).

Values $z$ were determined by the dynamic method given in para. 1.7 on the basis of the anisothermal survival curves. A typical anisothermal survival curve can be seen in Fig. 5.

Calculations of the data of the thermal death curve, in the case of heat treatments at various temperatures are given in Table 1.

Points of the thermal death curve were obtained by plotting the logarithmic values of the decimation times as a function of the temperatures (Fig. 6).

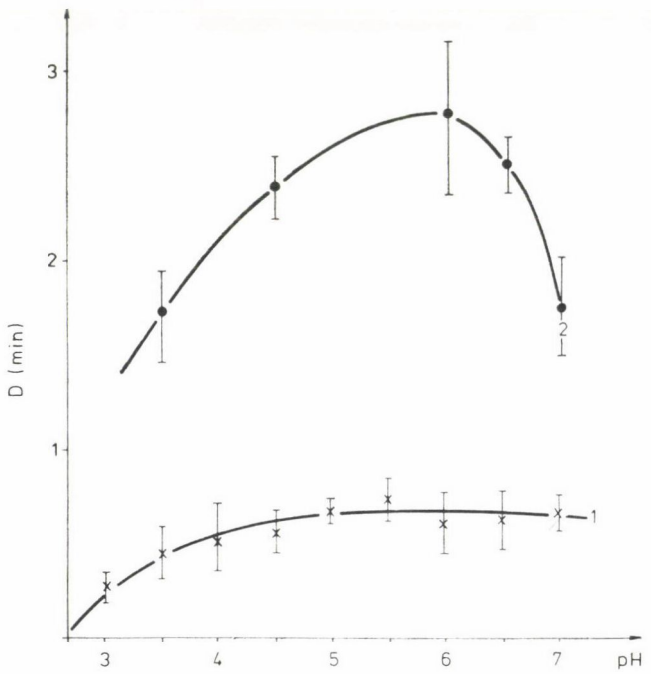



Fig. 4. Decimal reduction times $D$ as a function of the pH. 1: less resistant organisms; 2: resistant organisms

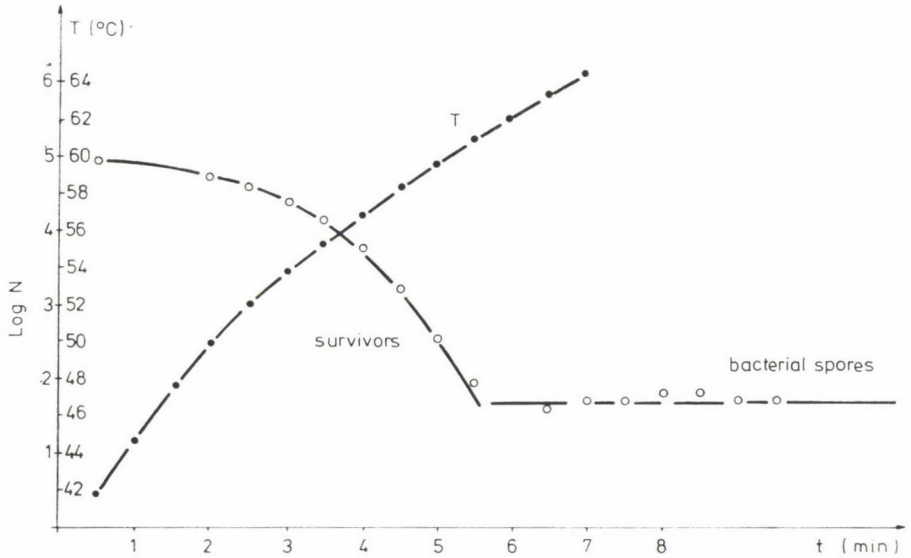



Fig. 5. Anisothermal survival curve. pH = 4.6; $D_{60\,°C}$ = 0.628 min

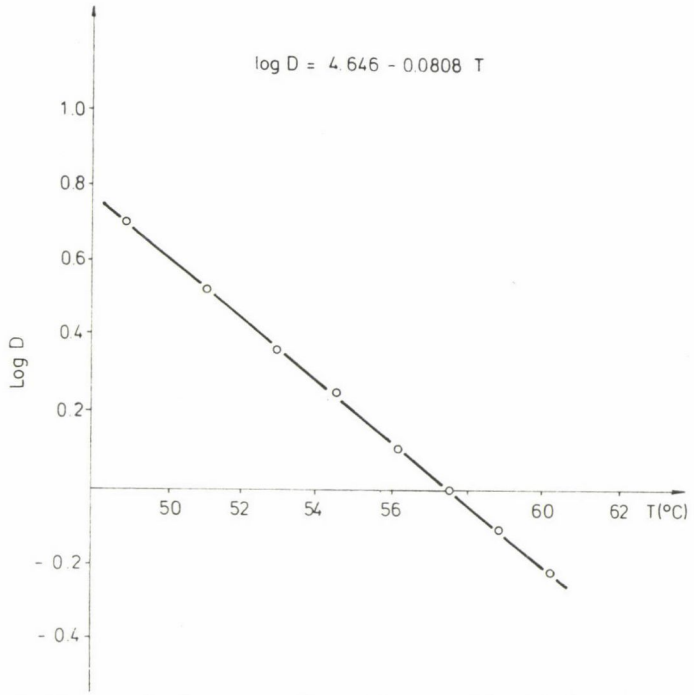



Fig. 6. Anisothermal death curve. pH = 4.6, $z$ = 12.37 °C; $D_{60\,°C}$ = 0.628 min

The equation of the heat destruction curve was determined by linear regression and the negative reciprocal value of the slope of the straight line yielded the value of $z$ (Fig. 6).

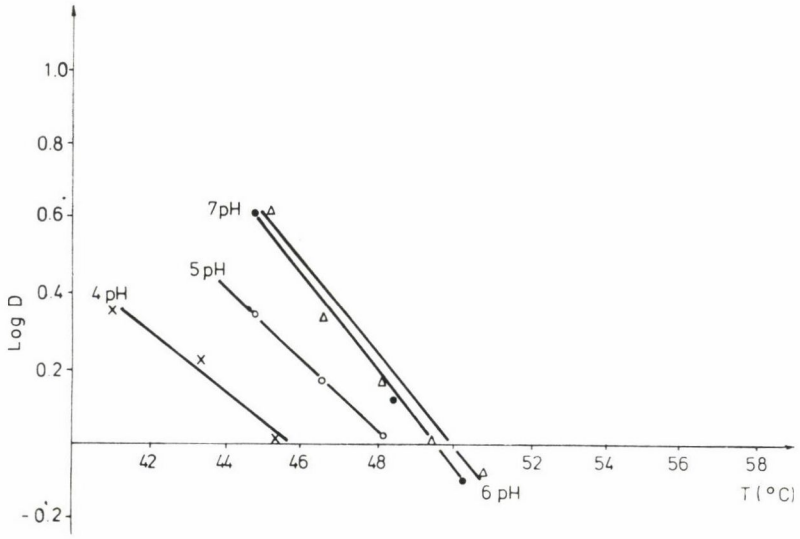



Fig. 7. The $z_1$ section of the death curves, measured at various pH

Table 1

*Calculation of the data of thermal death curve in the course of heat treatment at different temperatures*

| Time (min) | $T$ (°C) | $T_i$ (°C) | log $N_i$ | $-\frac{d \log N}{d_i} = k'$ (min$^{-1}$) | $D = \frac{1}{k'}$ (min) | log $D$ |
|---|---|---|---|---|---|---|
| 0 | 40 | | 4.95 | | | |
| | | 40.9 | | 0.04 | 25.00 | 1.398 |
| 0.5 | 41.8 | | 4.93 | | | |
| | | 43.3 | | 0.06 | 16.70 | 1.222 |
| 1.0 | 44.7 | | 4.90 | | | |
| | | 46.2 | | 0.10 | 10.00 | 1.000 |
| 1.5 | 47.7 | | 4.85 | | | |
| | | 48.9 | | 0.20 | 5.00 | 0.699 |
| 2.0 | 50.0 | | 4.75 | | | |
| | | 51.0 | | 0.30 | 3.33 | 0.523 |
| 2.5 | 52.0 | | 4.60 | | | |
| | | 52.9 | | 0.44 | 2.27 | 0.357 |
| 3.0 | 53.8 | | 4.38 | | | |
| | | 54.6 | | 0.56 | 1.79 | 0.252 |
| 3.5 | 55.3 | | 4.10 | | | |
| | | 56.1 | | 0.80 | 1.25 | 0.097 |
| 4.0 | 56.8 | | 3.70 | | | |
| | | 57.6 | | 1.00 | 1.00 | 0.000 |
| 4.5 | 58.3 | | 3.20 | | | |
| | | 58.9 | | 1.30 | 0.77 | −0.114 |
| 5.0 | 59.5 | | 2.55 | | | |
| | | 60.2 | | 1.66 | 0.60 | −0.220 |
| 5.5 | 60.9 | | | | | |

$T$: temperature of heat treatment
$T_i$: flash temperatures determined by interpolation, at time $i$
log $N_i$: logarithmic values of the number of survivors determined from the survival curve
$k$: constant of death rate
$D$: decimation time
log N: number of microbes

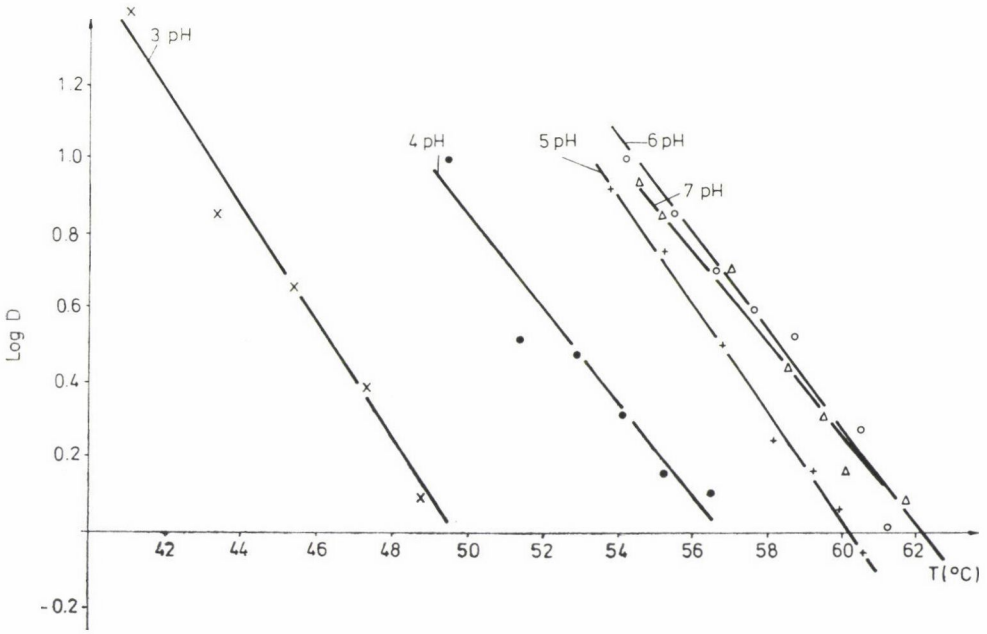



Fig. 8. The $z_2$ section of the death curves, measured at various pH

As a next step of the experiment, the relationship of pH and $z$ values was tested in the pH range of 3 to 7. Death curves belonging to different pH values — similarly to the isothermal survival curves — divided into two parts. The evaluation has been performed by comparing the values $z_1$ and $z_2$ measured in the first and second part of the curve, respectively.

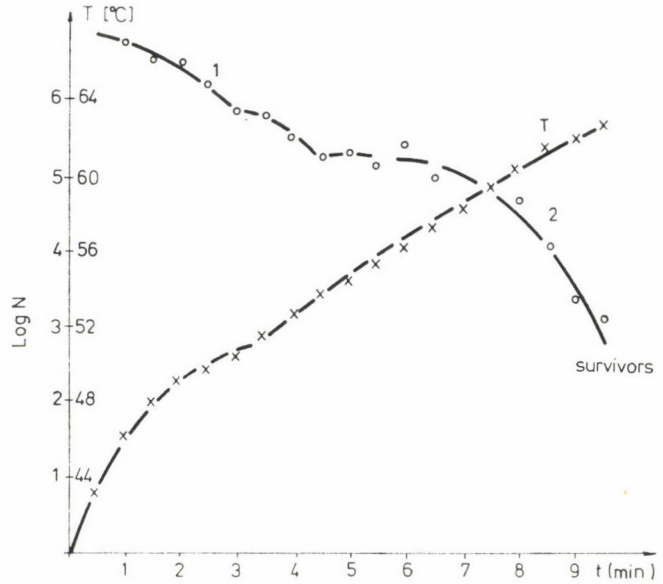
Correlation between the $z$ values and the pH did not prove as close as in the case of the $D$ values and pH. Values $z_1$ varied between 7.6 and 12.4 °C, while values $z_2$ were found to be in the temperature range of 6.3–8.1 °C (Figs. 8 and 9).

Data of death curves of the resistant fraction of the microflora (used for planning the rate of thermal death) were evaluated by analysis of covariance. It was found, that the slopes of the death curves did not change significantly as a function of the pH, consequently the thermal death of the microflora of the investigated samples can be characterized by the same $z$ value.

### *2.3. Determination of the necessary heat treatment*

The necessary heat treatment was planned on the basis of thermal death parameters of the experimental samples.

Thermal death curves of two fibrous carrot juices and a carrot-apple cocktail and the calculated values are shown in Figs. 9, 10 and 11, respectively.



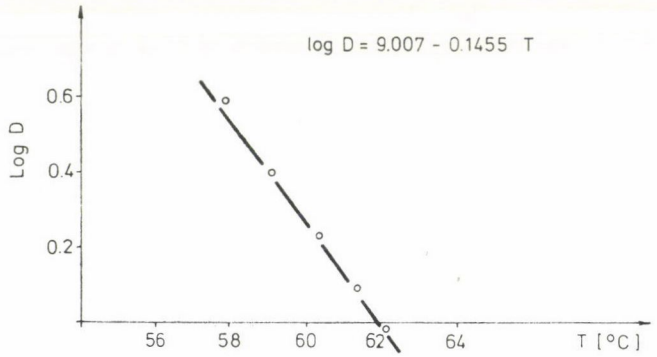




Fig. 9. Survival and death curves of the microflora in the first carrot juice series (I.) 1: less resistant; 2: resistant part of the microflora. pH = 5.45; $z$ = 6.87 °C; $r$ = —0.999

Table 2

*Calculated necessary heat treatment of soft drinks*

| Sample | pH | Equation of thermal death curve | $T$ (°C) |
|---|---|---|---|
| I. carrot nectar | 5.45 | $\log D = 9.007–0.1455\ T$ | 71.4 |
| II. carrot nectar | 4.80 | $\log D = 13.319–0.2139\ T$ | 68.7 |
| C–A cocktail | 4.20 | $\log D = 18.898–0.3181\ T$ | 63.8 |

$T$: temperature (°C) of heat treatment

The temperature needed (with 0.5 min holding period) to get a death rate of 12 orders of magnitude of destruction, as required in the canning industry, was calculated on the basis of the experimental results. Results are summarized in Table 2.

On the basis of the calculated values, the chosen parameters of heat treatment were the following: 65, 75, 85 and 95 °C.

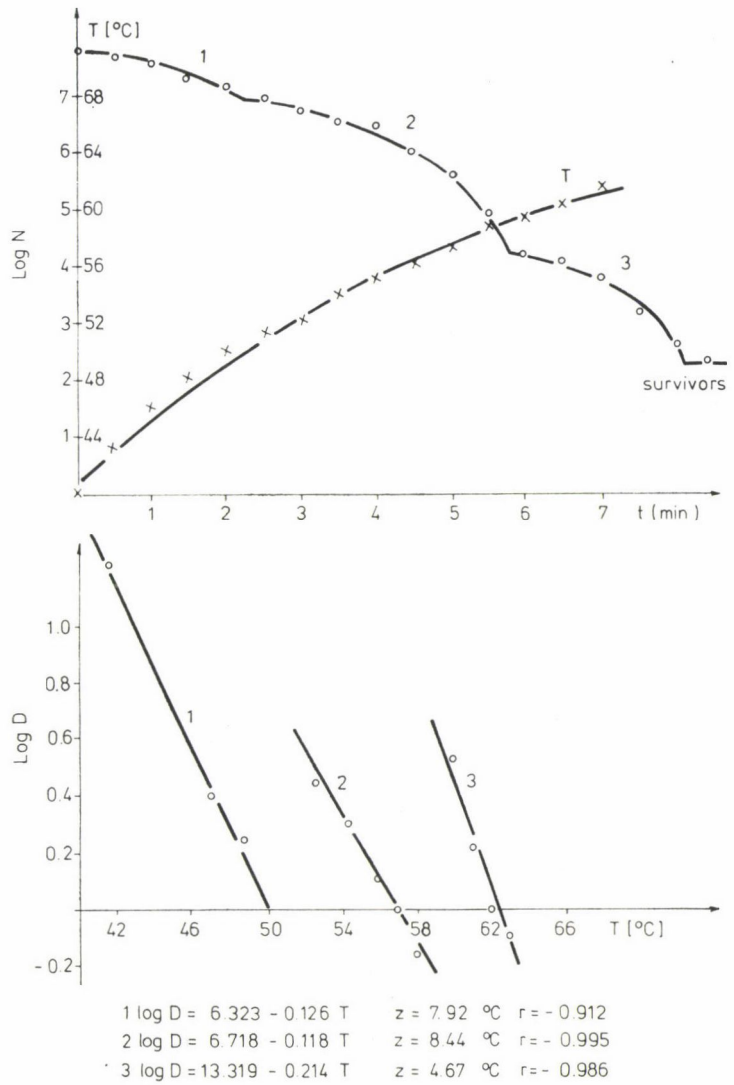



Fig. 10. Survival and death curves of the microflora in the second carrot juice series (II) 1: less resistant; 2: resistant and 3: most resistant part of the microflora. pH = 4.8

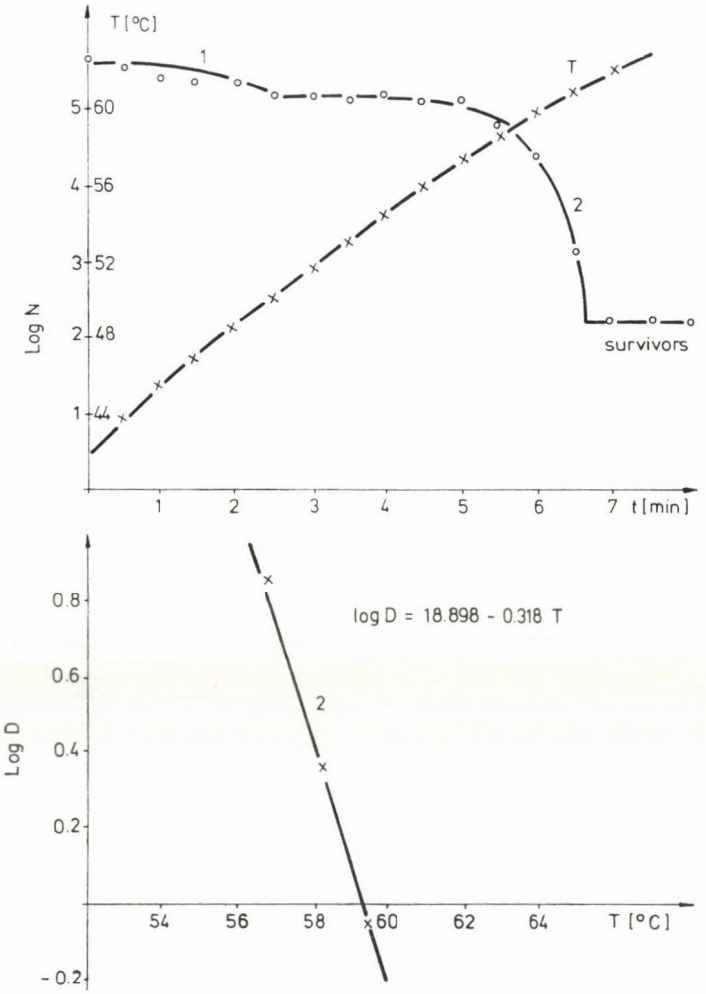



Fig. 11. Thermal death parameters of the microflora in the carrot-apple cocktail. 1: less resistant; 2: resistant part of the microflora. pH = 4.2; $z$ = 3.14 °C; $r$ = —0.998

## *2.4. Results of the storage experiments*

To support our results, a storage experiment was carried out in the course of which in addition to the microbiological state the vitamin content and the organoleptic properties of the samples were also tested.

Parameters of the storage experiments are given under section 1.6.

The number of microbes (log N) in the first series of carrot juice (I) increased from log 1.48–2.49 to log 6.0, while the juices deteriorated. The cause of the rapid deterioration was the initial pH (the value of which exceeded the critical value of pH 4.5) and this made possible the germination of the residual spores (Fig. 12).

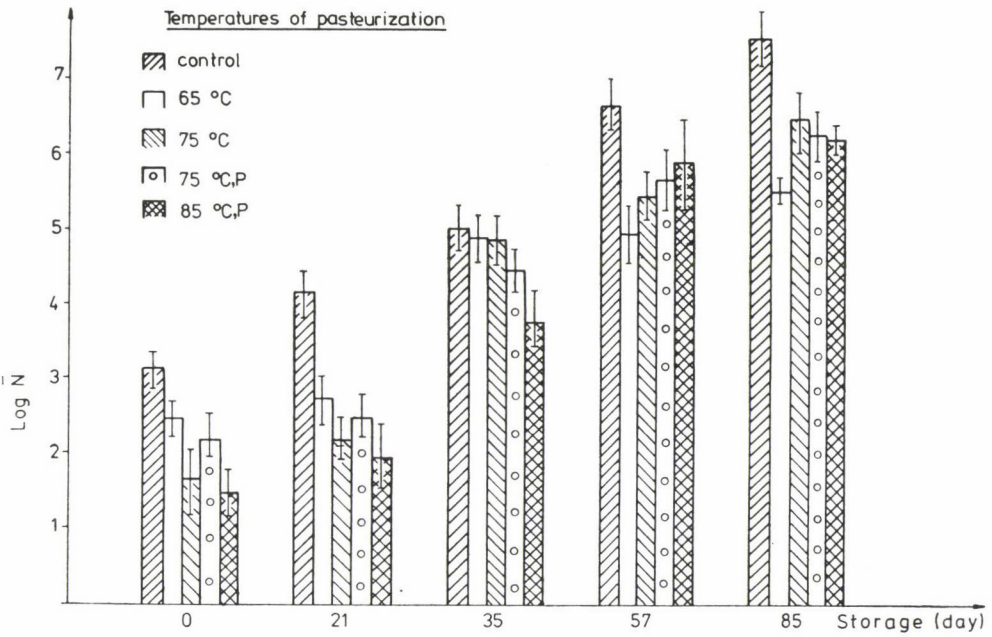



Fig. 12. Change in the number of the residual spore forming microorganisms as a function of the storage time. Temperature = 4 °C; initial pH = 5.45. *P:* recooled immediately after pasteurization

The second carrot juice series (II) and the carrot-apple cocktail were heat treated at a suitable initial pH. Consequently satisfactory results were obtained in the storage experiment applying the same heat treatment as with the first series (I).

The applied heat treatment ensured manifold protection against vegetative forms of microorganisms and against yeasts and moulds, while against the residual spore-forming organisms the values of pH proved to be the most effective. The tested samples maintained microbiological stability during a 6 months storage period.

Good results of the investigations of the nutrients, carotene content, colour changes (instrumental method) and organoleptic properties of the stored samples supported the above findings.

## 3. Conclusions

On the basis of the microbiological investigations of soft drinks, important results were achieved.

The thermal death of the microflora of the vegetable drinks can appropriately be characterized by the survival and the death curves, respectively.

Reflecting the heat resistance of microorganisms the curves obtained had mostly two sections. The heat treatments were always planned for the microflora of the highest resistance.

In the knowledge of the thermal death parameters a heat treatment of 0.5 minute at 80 °C seems to be suitable for the preservation of the nutrients, vitamins, colour and the organoleptic properties of the drinks.

However, it must be taken into consideration that this heat treatment gives protection only against the vegetative forms of bacteria and against yeasts and moulds.

The most suitable way of protection against spore-forming bacteria is to keep the pH of the drinks below the critical level (pH 4.5). At pH values higher than 4.5, the heat treatment must be higher than 100 °C, planned for the spore-forming bacteria, in accordance with hygienic requirements.

To prevent re-infection the aseptic technology can be suggested.

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# ASSAY INTO THE CORRELATION BETWEEN PROTEIN COMPOSITION AND BAKING QUALITY OF WHEAT FLOURS

E. PALLAGI-BÁNKFALVI and F. ÖRSI

College of the Food Industries, H-6724 Szeged, Marx tér 7. Hungary
Department for Biochemistry and Food Technology, Technical University, Budapest, H-1111 Budapest, Műegyetem rkp. 3. Hungary

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It is very important to investigate from both the aspect of theory and practice the characteristics of wheat proteins and the baking quality of flour milled from the wheat. The problems as yet unresolved weigh heavily on the industry engaged in complex processing of wheats of different varieties. Protein fractions of six wheat varieties were fractionated by various methods and the protein spectrum as obtained by SDS-PAGE gel electrophoresis, was studied. The amounts of the fractions and the differences among them unambiguously characterize the varieties. The mathematical statistical evaluation of the results by methods of multi-variable biometry remains to be accomplished.

**Keywords:** wheat protein, fractionation with eluents, GEP analysis of principal components

The quality of wheat flour and of the bread and other products of the baking industry, prepared from it, is highly affected by the quantity and quality of the flour components. Studies into the relations between protein composition and baking quality of flour were not very successful so far and produced contradictory results. One of the causes of this lies with the circumstance that the various methods of fractionation produce chemically and biologically different protein fractions. The separation of the low and high molecular mass protein fractions is particularly difficult. They are not easily soluble and may undergo chemical changes during dissolution, while complex proteins such as glyco- and lipoproteins are formed (LÁSZTITY, 1981).

The quality of the fractions depends also on the quantity of the fractions and their interrelations (LÁSZTITY, 1972).

The diverse correlations of the numerous components, dependent largely on variety, year of growth and methods of analysis may be responsible for distortions in the actual correlations.

In the course of these investigations it was attempted to increase the number of variables and apply mathematical statistical methods which enable to find the background variables responsible for true correlations, however, directly not analysable.

This first part of the study contains the methods of fractionation and analysis. In the second part correlations are detected by principal component or factor analysis.

## 1. Materials and methods

### *1.1. Materials*

Six wheat cultivars grown at the Cereal Research Institute (Szeged, Hungary) were studied. These are shown in Table 1.

Table 1

*Wheat varieties studied*

| Wheat variety | Strain |
|---|---|
| Yubileinaya 50 | |
| GK-F2 | Triticum |
| Rana 1 | Aestivum |
| Sava | |
| Partizanka | |
| GK-Basa | T. durum |

Subsequent to conditioning the samples were milled on a Quadromat (Senior Brabender) laboratory grinding apparatus. The flour fraction below 150 $\mu$m (about 60% of the flour) was used for the experiments. Three groups of analysis were carried out.

### *1.2. Methods*

*1.2.1. Determination of baking quality.* Standard methods as used in the baking and pasta industries (KARÁCSONYI, 1970), were applied.

Total protein content according to Kjeldahl, the quantity of moist and dry gluten and spreading of the gluten were established. Valorigraphy was applied to establish the water absorption capacity, dough formation and stability periods and the extent of softening. Volume of the loaf and the form quotient were determined by baking test. Results of these experiments have been published in an earlier paper (PALLAGI-BÁNKFALVI, 1984).

*1.2.2. Analysis of the composition of the flour samples.* To characterize the protein content of the flours three methods of fractionation were used. The quantity of the fractions was established by the modified biuret reaction.

Schematic diagrams of the fractionation procedures are shown in Figs. 1, 2, 3 and 4.

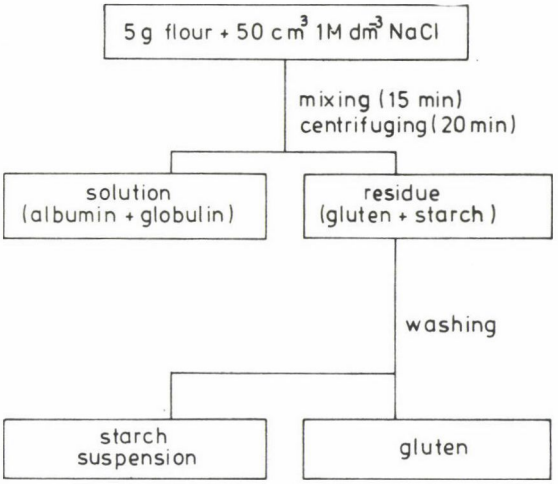


Fig. 1. Production of the gluten complex

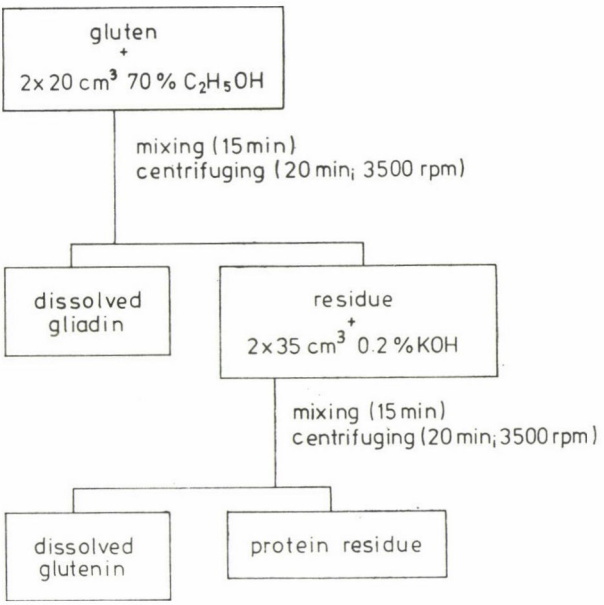


Fig. 2. Fractionation of gluten proteins by the modified Osborne method

In the first step the salt-soluble fractions were extracted and the gluten complex was obtained. In order to prevent the dissolution of the starch and thereby causing a disturbing effect, it was tried to wash out the gluten as thoroughly as possible.

Prior to fractionation according to Osborne the flour was defatted. Ten g of the flour were suspended in 200 cm³ petroleum ether and mixed for

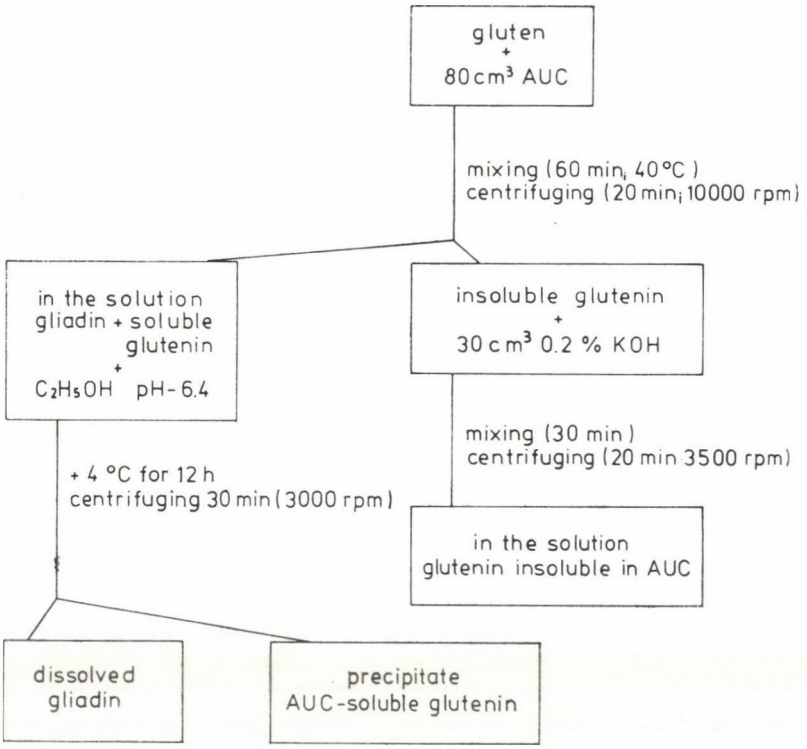


Fig. 3. Fractionation of gluten proteins using AUC solving agent. (AUC = 0.1 *M* acetic acid, 3 *M* urea, 0.01 *M* cetyltrimethylammonium bromide)

30 min in a magnetic blender. After sedimentation the petroleum ether phase was removed. The process was twice repeated, then the defatted flour was dried at room temperature. Five g of the product were weighed in and used for gluten complex production. Centrifuging was carried out in a Janetzki T 30 type apparatus.

The gluten was washed out in tap water. The blobs of gluten thus obtained were cut in small pieces in the case of all three fractionation methods.

In the knowledge of the solubilities of the protein fractions the fractionation procedures were planned to obtain possibly characteristic fractions using the most important procedures in appropriate combinations.

Each method of fractionation was repeated three times. In the Tables the averages and the standard deviations are presented. The standard deviations were averaged related to the flour varieties because significant differences were not observed.

*1.2.3. Gel electrophoresis of the gluten fractions.* Of the gluten fractions separated by their different solubilities, glutenin according to Osborne, AUC-

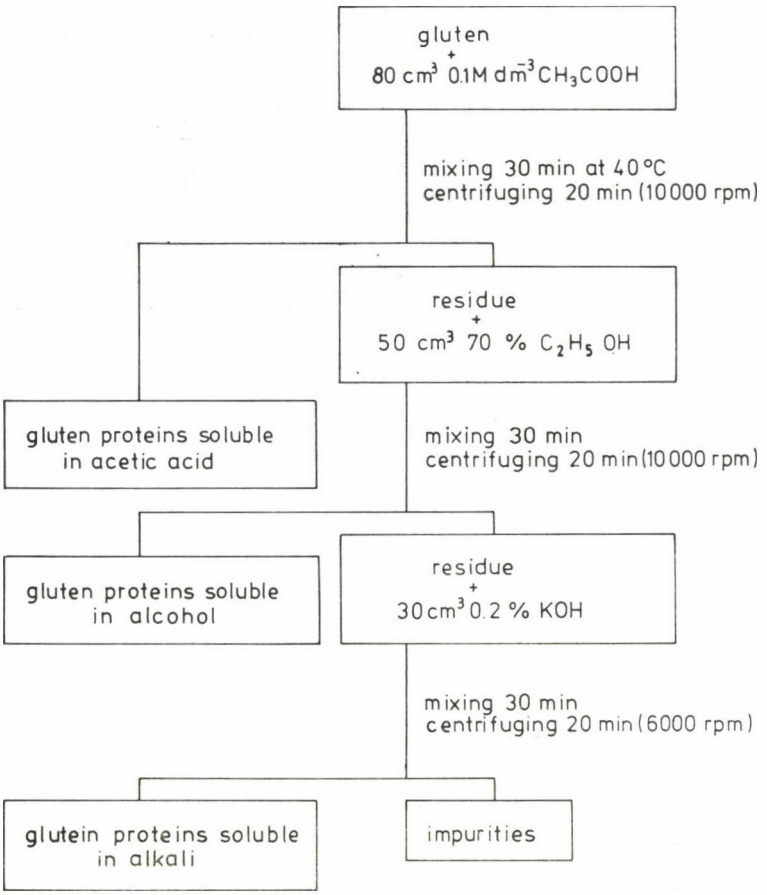


Fig. 4. Fractionation of gluten proteins using 0.1 *M* acetic acid

soluble and insoluble fractions and the fraction soluble and insoluble in 0.1 *M* acetic acid solution were purified by dialysis in a refrigerator of +4 °C for 48 h against distilled water and finally freezedried. Lyophilization was carried out in a DE–950 type equipment (manufactured by Labor MIM, Hungary) at −30 °C and 0.13 Pa pressure for 24–30 h. The samples were stored in a desiccator.

To carry out gel electrophoresis of protein fractions it is necessary to reduce the high molecular mass proteins and to produce SDS (sodium dodecyl sulfate) complex. Ten g of each of the purified and lyophilized fractions were weighed and incubated for 12 h in an electrode buffer containing 1 cm³ 1% SDS and 2% $\beta$-mercaptoethanol at 40 °C. After reduction with mercaptoethanol the SDS-PAGE test was carried out, using the buffer systems according to LAEMMLI (1970).

Solution used in electrophoresis:

- Gel solution: acrylamide, 29.2 g; N,N,N',N'-methylenebisacrylamide, 0.8 g, made up to 100 $cm^3$ with distilled water.
- Gel buffer I: 1 *N* hydrochloric acid, 48.0 $cm^3$; hydroxy-methylamino-methan, 36.6 g, made up to 100 $cm^3$ with distilled water; pH: 8.8.
- Gel buffer II: 1 *N* hydrochloric acid, 48.0 $cm^3$; hydroxy-methylamino-methan, 5.9 g; made up to 100 $cm^3$ with distilled water; pH: 6.8.
- Sodium dodecyl sulfate (SDS) solution: SDS 10.0 g in 100 $cm^3$ distilled water.
- Ammonium persulfate solution: ammonium persulfate 10.0 g in 100 $cm^3$ distilled water.
- N,N,N',N',-tetramethyl-ethylene diamine (TEMED).
- Electrode buffer: Tris(hydroxymethyl)aminomethan 30.2 g; glycine 144.2 g; SDS 10.0 g made up to 1000 $cm^3$ with distilled water; pH: 8.3. Used in ten-fold dilution with distilled water.

The electrophoresis was carried out in a vertical plate electrophoresis apparatus at 50 mA. The gel solutions were obtained by mixing the components as listed in Table 2.

Table 2

*Gel composition in electrophoresis*

| Gel components | Separating gel ($cm^3$) | Collecting gel ($cm^3$) |
|---|---|---|
| Gel solution | 6.70 | 3.34 |
| Distilled water | 5.50 | 13.86 |
| Gel buffer I | 7.50 | 0.00 |
| Gel buffer II | 0.00 | 2.50 |
| SDS solution | 0.20 | 0.20 |
| Ammonium persulphate | 0.10 | 0.10 |
| TEMED | 0.01 | 0.01 |
| Final volume | 20.00 | 20.00 |

The analysis of the standard deviation gave homogeneous distributions. The second powers of scatters of fractionation methods are given in the last row of Table 3 as characteristics. While two degrees of freedom belong to the individual standard deviations, to the average standard deviation belong 12. This helps substantially avoiding the subordinate error in the statistical tests applied to compare the average values. Since loss in the information content is not caused by the averaging of the homogeneous variances, thus, there is no point in presenting the standard deviation of each of the three parallels. The degree of freedom belonging to the standard deviations is 12.

Table 3

*Quantity of protein fractions prepared from the flour samples as a percentage of the protein in the flour*

| Wheat variety | Methods of fractionation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Salt-soluble fraction (%) | Gliadin fraction (%) | Glutenin fraction (%) | AUC-soluble fraction (%) | 0.2 *M* KOH-soluble fraction (%) | 0.1 *M* acetic acid-soluble fraction (%) | 70% ethylalcohol soluble fraction (%) | 0.2 *M* KOH-soluble fraction (%) |
| Yubileinaya 50 | 14.17 | 30.70 | 47.87 | 40.04 | 45.00 | 34.70 | 9.20 | 38.40 |
| GK-F2 | 14.81 | 30.30 | 48.16 | 35.40 | 49.70 | 39.00 | 11.20 | 30.50 |
| Rana 1 | 14.30 | 33.16 | 46.34 | 43.80 | 37.90 | 40.00 | 18.90 | 24.20 |
| Sava | 14.60 | 40.20 | 42.83 | 47.70 | 37.30 | 43.70 | 25.90 | 13.00 |
| Partizanka | 13.60 | 38.83 | 44.18 | 32.60 | 53.20 | 35.40 | 15.50 | 30.90 |
| GK-Basa | 15.90 | 52.32 | 30.89 | 59.10 | 24.00 | 50.70 | 22.30 | 6.60 |
| Scatter | 0.43 | 0.38 | 0.40 | 0.39 | 0.46 | 0.42 | 0.65 | 0.34 |

Prior to adding the catalyzer the solutions were de-aerated with a water-jet pump. At room temperature polymerization took 20 min. When the separating gel was polymerized the collecting gel was layered on the top and in this was placed the element forming the sample place. The gel plates prepared from the gel solutions were of $12 \times 14$ cm size and 2 mm thickness (4 cm collecting gel + 10 cm separating gel). In each of the sample places 20 $mm^3$ reduced SDS protein was applied. Bromophenol blue was used for staining. Electrophoresis was carried on till the stain was removed by 10 cm.

To fix the protein stripes the gel plates were immersed for 30 min in a 12.5% trichloroacetic acid solution.

Staining was carried out at room temperature with Coomassie Brillant Blue R-250 solution. The solution contained 454 $cm^3$ 50% methanol + 46 $cm^3$ acetic acid + 0.8 g Coomassie Brillant Blue R-250.

In order to be able to measure the migratory distance of the protein zones the background has to be bleached. Composition of the bleaching solution: 50 $cm^3$ methanol + 75 $cm^3$ glacial acetic acid + 875 $cm^3$ distilled water. The gel plates were immersed for 24 h in the bleaching solution. The solution was several times exchanged till a well differentiated gel pattern was obtained.

To evaluate the gel plates a Chromoscan (Joyce Loebl and Co, Ltd., England) densitometer was used. The densitograms were taken at 620 nm and 0.5 mm partial width.

To estimate the molecular mass of the polypeptide chains separated by SDS-PAGE a calibration line was prepared. The calibration line was prepared on the basis of 8 protein preparations of analytical purity, the products of Pharmacia Fine Chemicals. Proteins and molecular masses belonging to the reference points can be seen in Fig. 5.

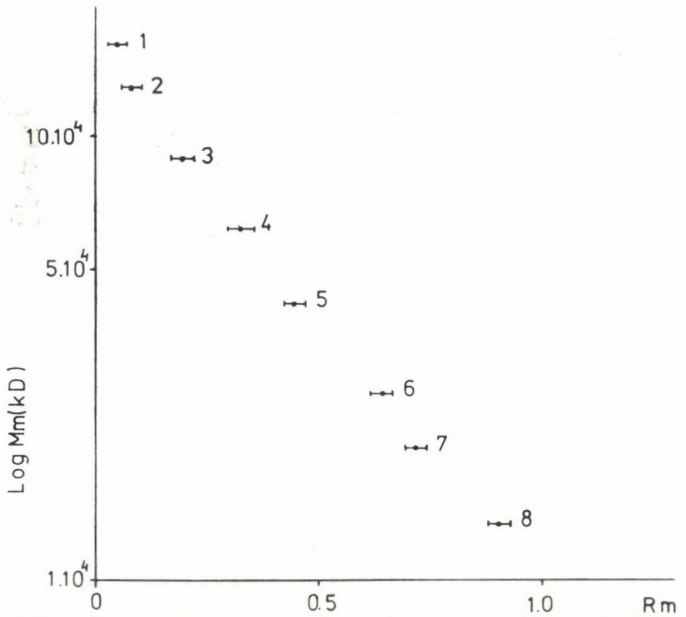


Fig. 5. Calibration line to estimate the molecular mass of polypeptides separated by SDS-PAGE. ($Mm$ = molecular mass, $Rm$ = relative mobility related to bromophenol blue) 1 = ferritin $Mm$ = 220 kD; 2 = phosphorylase $Mm$ = 94 kD; 3 = albumin $Mm$ = 67 kD; 4 = catalase $Mm$ = 60 kD; 5 = ovalbumin $Mm$ = 43 kD; 6 = lactate-dehydrogenase $Mm$ = 36 kD; 7 = trypsin inhibitor $Mm$ = 20.1 kD; 8 = α-lactalbumin $Mm$ = 14.4 kD

## 2. Results

Results are presented in two parts. In the first part the results of the quantitative determinations of protein fractions are discussed. The quantities of protein fractions separated from flours judged to be of better or poorer quality on the basis of baking tests, were compared.

In the second part the results of the electrophoretic analyses are discussed.

### *2.1. Quantitative analyses of the protein fractions*

The quantities of the protein fractions separated by different fractionation techniques from the flour studied are presented in Table 3. Differences were found in the amounts of protein fractions.

The lowest amount of salt-soluble protein was found in the wheat variety Partizanka. The highest amount was found in the durum wheat GK-Basa. Practically no difference was observed in the quantity of the fractions obtained from the other four wheat varieties.

Contrasting differences were found in the quantity of the gliadin and glutenin proteins as obtained by Osborne fractionation. In the cultivars Yubileinaya 50 and GK-F2 was found the highest amount of glutenin, while the lowest amount of gliadin. At the other end of the series in cultivar GK-Basa the reverse amounts were found. In the flours of varieties Rana 1, Partizanka and Sava the fractions were found between the two limit values and did not differ. Differences are substantially more striking if the ratios of the two fractions are compared: in cultivars Yubileinaya 50 and GK-F2 the ratio is 1 : 6; in Rana 1 1 : 4; in Partizanka 1 : 0 and in GK-Basa 0 : 6.

The protein content soluble in AUC was found to be substantially lower than given in the literature. In the literature Canadian autumn wheats were described to show solubility of 90%. In the present study the lowest value was found for Partizanka: 33% and 35% for GK-F2, 40% for Yubileinaya 50, 44% for Rana 1, 48% for Sava. The highest solubility value was found in the cultivar GK-Basa and this was 60%.

The alcohol-soluble fraction of gliadin character increased in the same order: 21% for GK-F2 and Partizanka, while 40% in the GK-Basa cultivar. In relation to the total AUC-soluble protein content these represent values below 70% for the varieties of higher baking quality (GK-F2: 59%, Partizanka: 64% and Yubileinaya 50:66%, respectively). In the cultivars of lower baking quality the value was above 70% (74% for Rana 1, 80% for Sava and 74% for GK-Basa, respectively).

The ratio of AUC-soluble and insoluble fractions show also contrasting difference. The lowest value was found for cultivar GK-Basa, while the highest for Partizanka. Varieties Rana 1 and Sava did not differ, while Yubileinaya 50 and GK-F2 showed higher values.

The fractions soluble in 0.1 *M* acetic acid showed a similar order to the AUC-soluble fractions, with the difference that here the lowest values were found for cultivars Yubileinaya 50 and Partizanka.

The fact that in the residue of the acetic acid solution a further 9–25% protein could be solubilized with 70% alcohol shows that the acetic acid-soluble fraction contains a substantial amount of glutenin fraction. Its quantity mostly reaches or exceeds the amount of gliadin as obtained by Osborne fractionation. This technique seems to be different in its character. This observation is supported by the fact that the amount of the insoluble fraction is substantially lower than the glutenin fraction according to Osborne or the AUC-insoluble fraction.

The lowest amount of acetic acid soluble fraction was found in cultivars Yubileinaya 50 and Partizanka, while the highest amount in GK-Basa. Cultivars GK-F2, Rana 1, and Sava are between the above limits with values increasing in the order as listed. A similar order is shown by the protein fractions insoluble in acetic acid but soluble in alcohol. Partizanka is in the middle

of the series, while Sava gave the highest results. The amounts of fractions soluble neither in acetic acid nor in 70% alcohol were lowest in GK-Basa followed in increasing order by Sava, Rana 1, Partizanka, GK-F2 and Yubileinaya 50.

As it can be seen from the results the three methods of fractionation yield fractions differing in quality and quantity.

In wheat varieties yielding flour of good baking quality the glutenin-gliadin ratio as obtained by Osborne fractionation is above 1 : 1, while the AUC- and alcohol-soluble gliadin fraction is below 70%.

In flours of good baking quality a ratio of about 1 is characteristic of the fraction soluble in 0.1 *M* acetic acid and that soluble in 0.1 *M* acetic acid and in 70% alcohol.

The protein fractionation methods studied seem to be suitable for the characterization of flours of different baking quality, however, the limit values needed to establish quality require the analysis of greater number of samples.

For the comparison of the quantity of fractions obtained from the same sample by different methods the correlation coefficients of the fractions were determined.

The correlation coefficient of $r = -0.98$ supports the observation of controversial change in the amounts of glutenin and gliadin according to Osborne. The acetic acid-soluble and insoluble fractions showed a controversial tendency with a correlation coefficient of $r = -0.92$, while the AUC-soluble and insoluble fractions had a correlation coefficient of $r = -0.999$.

The amount of the salt-soluble fraction is in positive correlation with the acetic acid-soluble fraction and in negative correlation with the glutenin fraction according to Osborne ($r = 0.88$ and $r = -0.83$, respectively). It is presumed that a part of the salt-soluble fraction goes into the gliadin fraction and increases its amount.

The authors consider this to be due to the difficulties int he solid–liquid extraction and find necessary to determine experimentally the time of extraction. However, the possibility must not be excluded, that the correlation observed in the quantity of the two fractions can be traced back to the plant-physiological mechanism of their formation. In other words the salt-soluble proteins in part are formed of the gliadin fraction by enzymatic action (wheat proteases).

The correlation between the Osborne glutenin on one hand and the acetic acid and alcohol insoluble on the other, further that between the AUC-soluble and alcohol-soluble fractions on one hand and the acetic acid-soluble fraction on the other, shows the presence of proteins of similar character and quantity. The negative correlation between the Osborne gliadin and the acetic acid-insoluble fraction can be motivated similarly. The negative correlation

of Osborne glutenin and the acetic acid-soluble fraction and the positive correlation between Osborne glutenin and the acetic acid-insoluble fraction shows the contrasting character of the changes of these fractions. The negative correlation between the alcohol-soluble and the acetic acid-insoluble fractions can be explained by similar reasons.

Correlations between different protein fractions are clearly shown by the principal component analysis, based on eigen-value calculations for the correlation matrix the result of which is illustrated in the space of the first two principal components. The vectors mark the direction of the axes expressing the quantities of the individual fractions and their size or projection is in proportion with their weight in the principal components. Gliadin type fractions and salt-soluble fractions (in support of former considerations) are directed in the direction of the values of principal component I. The glutenin type proteins are directed towards the negative part of the space. The acetic acid soluble and insoluble fractions are nearly coincidental with principal component I (Fig. 6).

The first two principal components contain more than 90% of the variance of variables, thus, they give a very good description of the system of

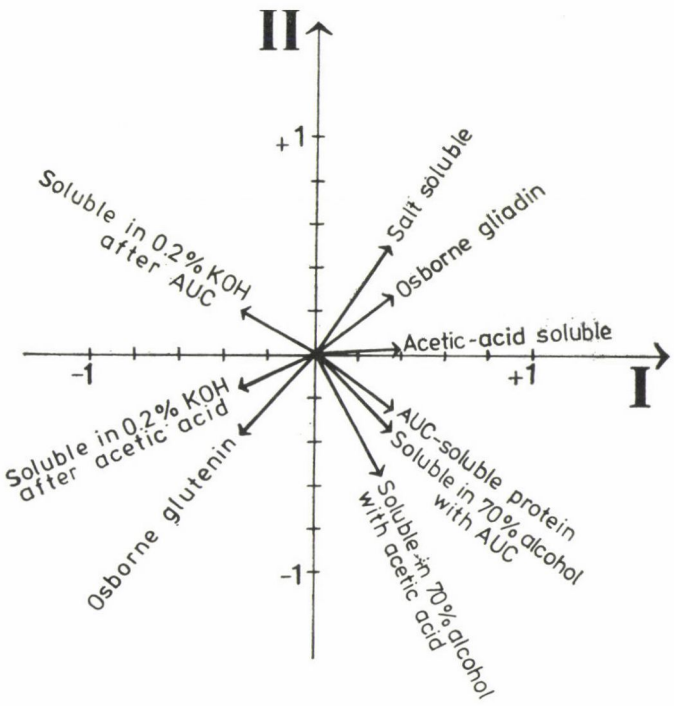


Fig. 6. Correlations of the different protein fractions in the plane of principal component I and II

correlations. It seems probable that the acetic acid-soluble and -insoluble fractions, nearly coincidental with principal component I, form a true picture of the complex system of wheat.

*2.2. Evaluation of the results of electrophoresis*

Figures 7 and 8 show the photograph of the gel plate obtained with setting the optimum parameters of electrophoresis. The Figures show the SDS-PAGE separation of the protein fractions of the flour samples studied, soluble and insoluble in acetic acid.

With the applied gel concentration and number of cross bindings the separated proteins fall in the range of 10–200 kD.

The electrophoretic spectra of protein fractions obtained from the flour samples are shown in Figs. 9–14.

The number of protein stripes is plotted as a function of mobility. Since under the analytical conditions the logarithm of the molecular mass of the protein fractions is in linear correlation with mobility a molecular mass scale was drawn on the mobility axis and the amounts of protein fractions were depicted by proportional lines.

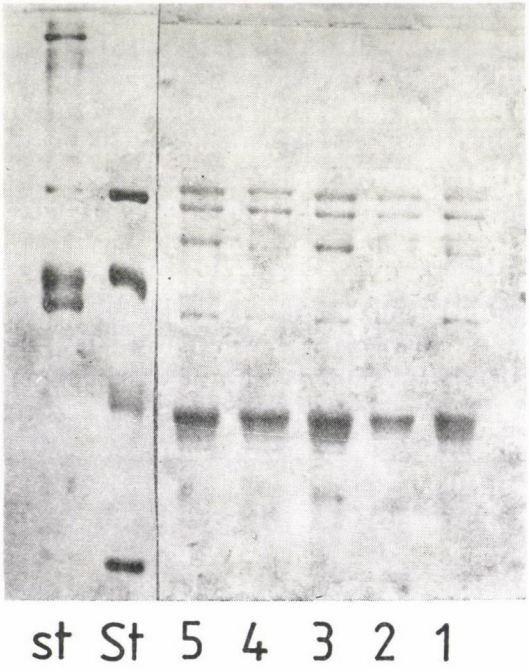


Fig. 7. The SDS-PAGE pattern of the 0.1 *M* acetic acid-soluble fractions. Wheat varieties: Sava (1), GK-F2 (2), Partizanka (3), Rana 1 (4), Yubileinaya 50 (5), St = standard of high molal weight, st = standard of low molal weight

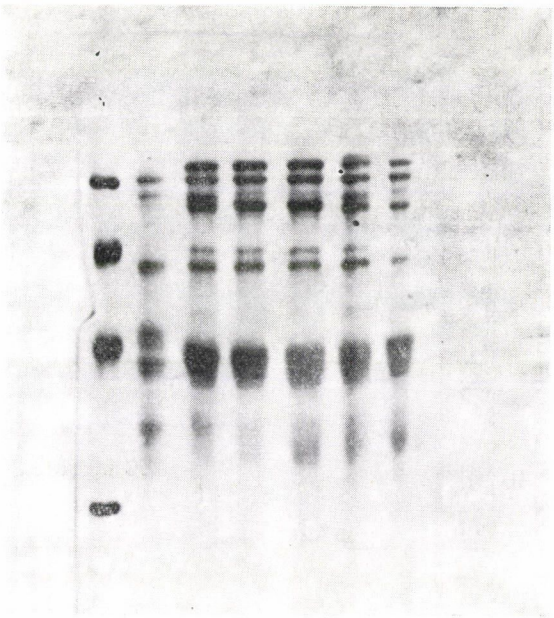


Fig. 8. The SDS-PAGE pattern of the 0.1 *M* acetic acid-insoluble fractions. Wheat varieties: Sava (1), GK-F2 (2), Partizanka (3), Rana 1 (4), Yubileinaya (5), Basa (6), St = standard

Subunits of the protein fractions were found in the range of 12.5 to 125 kD. As regards the number and molecular mass of the subunits substantial differences were found between protein fractions obtained by different fractionation techniques.

Subunits of lower molecular mass (below 71 kD) were characteristic of the acetic acid-soluble and AUC-soluble fractions. In the acetic acid-insoluble and AUC-insoluble fractions and in the glutenin according to Osborne subunits of higher molecular mass (above 40 kD) dominated.

Fractions obtained from identical flours and of identical character (e.g. glutenin fractionated according to Osborne, AUC- and acetic acid-insoluble fractions) considered identical contained subunits of different molecular mass and considerable differences were observed in their proportions, too. This is probably due to applying extracting solvents of different pH and different detergent contents thereby giving rise to differing interactions with the proteins present.

Quantitative evaluation of the electropherograms infers identical staining of the various subunits and proportionate amount of dye with that of

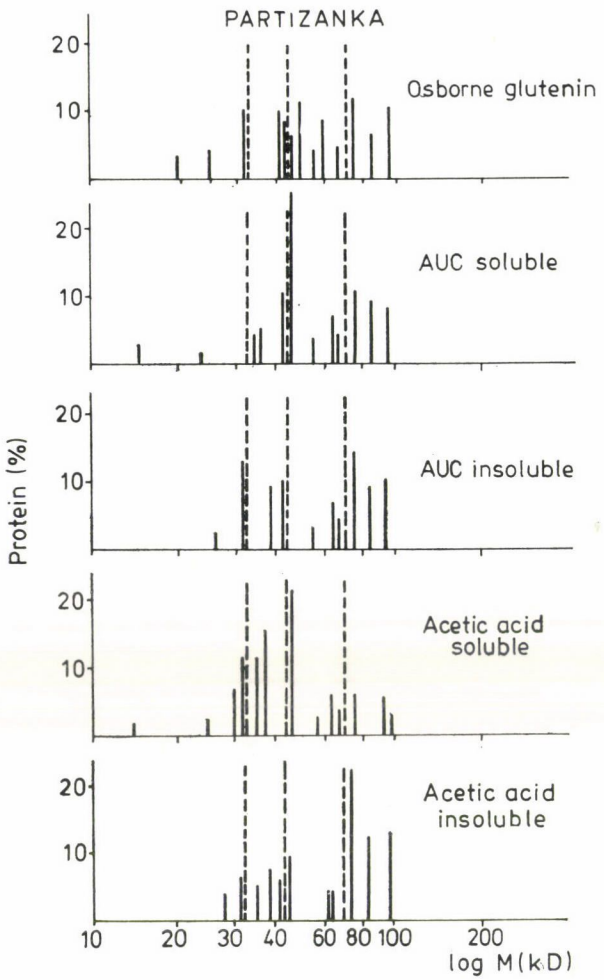


Fig. 9. The SDS-PAGE spectrum of the fractions obtained from cultivar Partizanka

the protein. To elucidate this question the acetic acid-insoluble fraction of the flour of variety Yubileinaya 50 was used. Of the solutions containing 2 to 12 mg $cm^{-3}$ of the fraction, respectively, 20 $mm^3$ volumes were applied and after electrophoresis the plate was fixed and stained and finally analysed with densitometer as described above.

In Fig. 15 the proportion of the peak heights, as seen in the densitogram of the main subunits, is plotted. The curves obtained increased monotonously with the amount of protein and these were grouped around the same curve.

The value $S$ as indicated is the relative error of the estimated value expressed in percentage and it was obtained as the scatter of log $y$ value when the logarithmic value was approximated by the least square method.

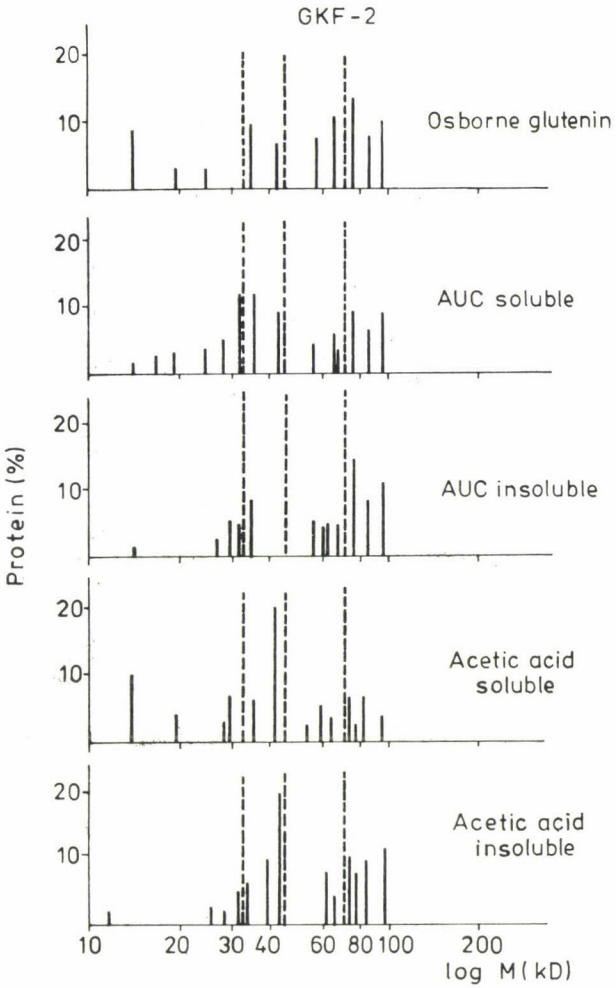


Fig. 10. SDS-PAGE spectrum of the fractions obtained from cultivar GK-F2

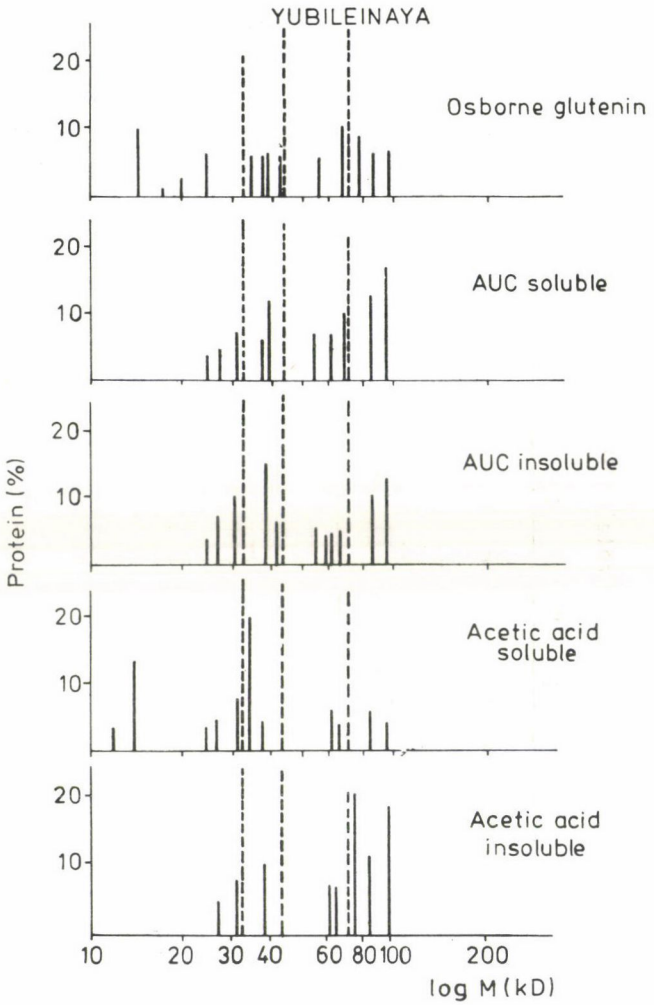


Fig. 11. SDS-PAGE spectrum of the fractions obtained from cultivar Yubileinaya 50

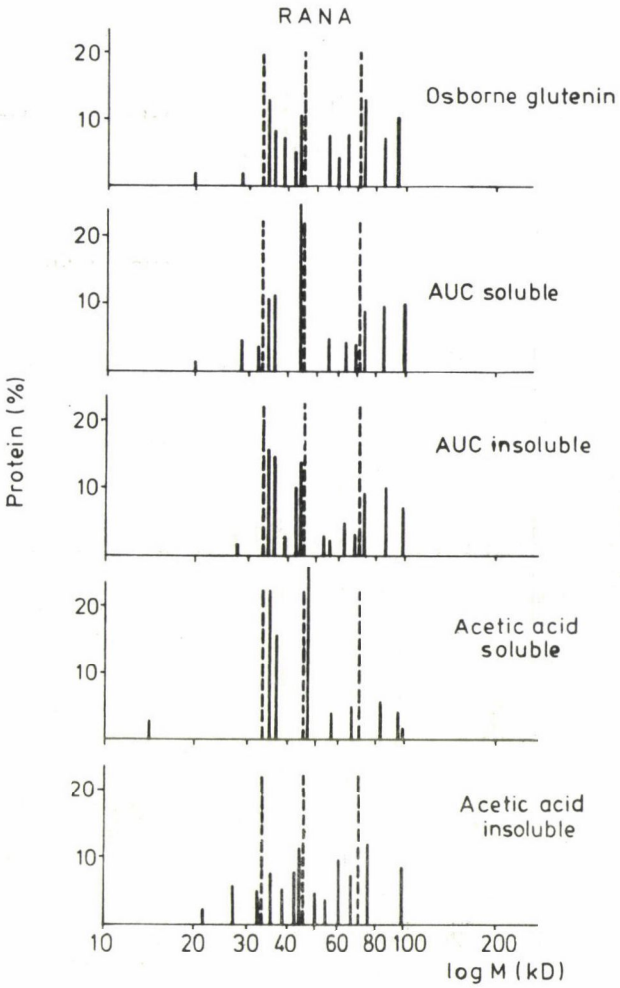


Fig. 12. SDS-PAGE spectrum of the fractions obtained from Rana 1

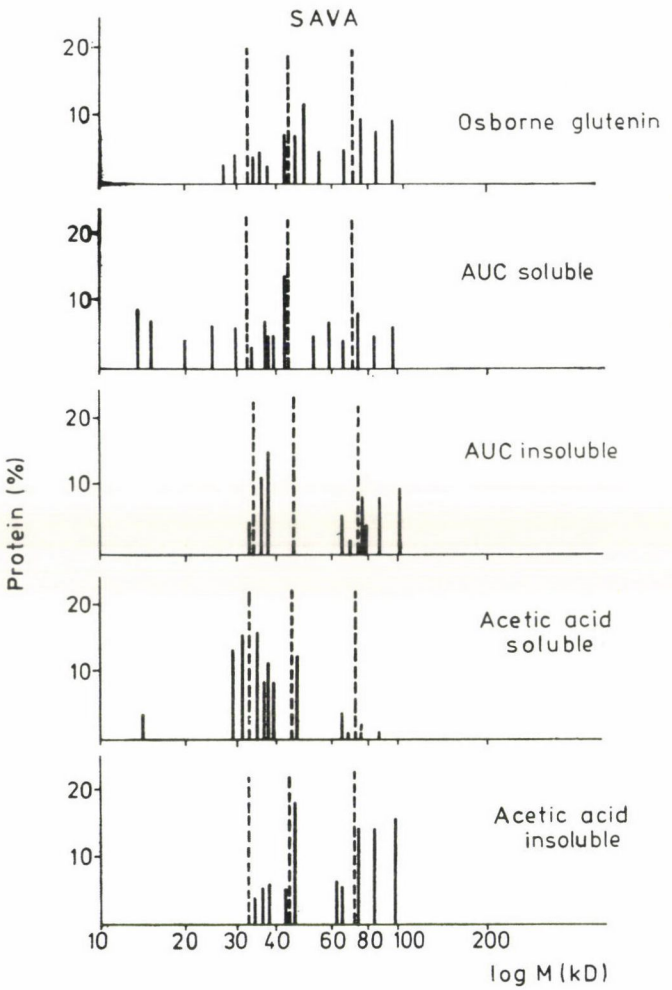


Fig. 13. SDS-PAGE spectrum of the fractions obtained from cultivar Sava

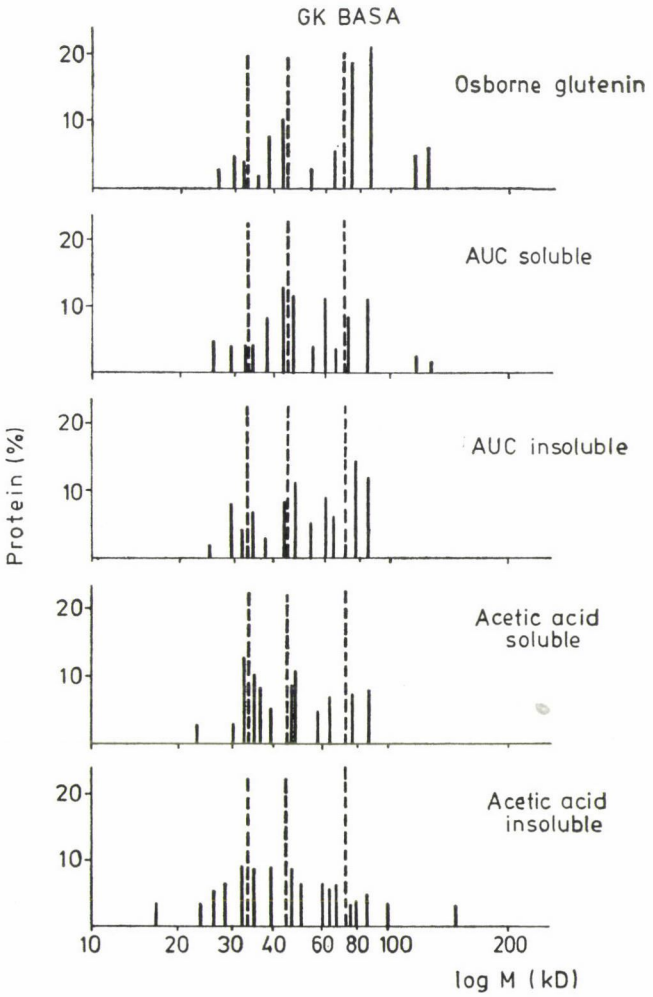


Fig. 14. SDS-PAGE spectrum of the fractions obtained from cultivar GK-Basa

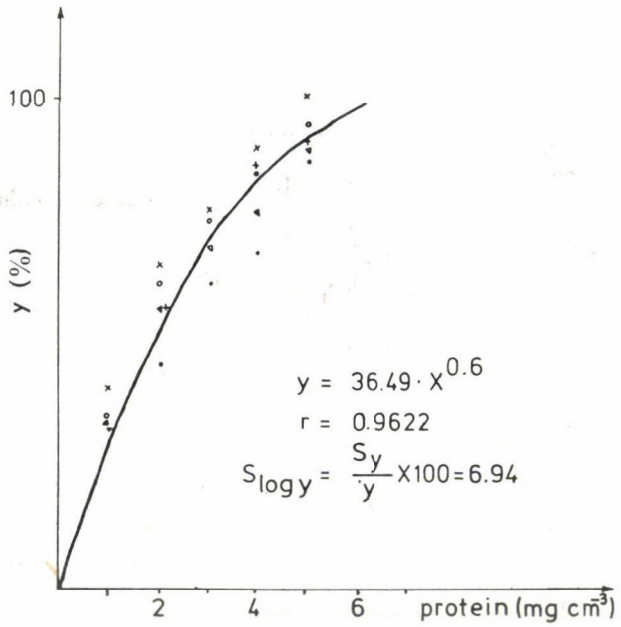


Fig. 15. Correlation between the concentration of the protein solution and the height of densitogram peaks

Thus, it can be concluded that quantitative evaluation in accordance with the presumed relationships can be carried out in the lower concentration range only and the value of fractions of different dye binding capacity may deviate from the actual, however, always in identical proportion. This does not bear on the conclusions drawn.

Analysis of the data of protein composition investigated from many aspects and of the results of quantitative evaluation of the electropherograms, of the informations contained in them is possible only by multi-variable methods of biometry. This will be discussed in an ensuing report.

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# ANALYSIS OF DEPENDENCE BETWEEN FLOUR QUALITY AND ELECTROPHORETIC PROTEIN SPECTRUM

F. Örsi[a], E. Pallagi-Bánkfalvi[b] and R. Lásztity[a]

[a] Biochemistry and Food Technology Department, Technical University, Budapest, H-1111 Budapest, Műegyetem rkp. 3. Hungary
[b] College of the Food Industries, H-6724 Szeged, Marx tér 7. Hungary

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To investigate the interrelations between the characteristics of wheat flour, the characteristics of bakery products made of it, the protein fractions in the flour and the quantities of subunits obtained by SDS-PAGE, the principal component correlation method was applied.

The quantities of the protein fractions obtained from the flour are influenced by the AUC-soluble subunits in the molecular mass range of 45 000–157 000, by the AUC-insoluble subunits in the molecular mass range of 74 000–159 000 as well as by the glutenin fractions in the molecular mass range of 12 500–33 000 as obtained by Osborne's method.

The properties of the dough and gluten made of the flour correlate with the quantities of subunits of the acetic acid-soluble fraction in the molecular mass range of 12 500–33 000, of the AUC-soluble fraction in the molecular mass range of 75 000–158 000, of the AUC-insoluble fraction in the molecular mass range of 45 000–74 000 as well as of the glutenin fraction in the molecular mass range of 46 000–72 000 obtained by Osborne's method.

The properties of bread are determined by the quantities of subunits of the acetic acid-soluble fraction and the AUC-insoluble fraction in the molecular mass range of 12 500–33 000.

**Keywords:** wheat protein, qualification of flours, SDS-PAGE analysis, factor analysis

The quality of flour, of the bread prepared from it and of the various kinds of pastries is determined by the quantity and quality of the protein components in the flour. In spite of the early recognition of these facts, apart from some general correlations, the role of the protein fractions obtained by electrophoresis in the quality of the products is not known.

One of the reasons for this is that the quantity of individual protein fractions and their interrelations as well as their relation to other flour components is also of great importance. The second difficulty lies in the intricacy of the correlation between the great number of components, depending largely on variety, year of growth, methods of analysis which can cover, deform the actual correlations.

In the course of this study an effort was made to increase the number of characteristics investigated and apply mathematical statistical methods which enable the discovery of variables responsible for the correlations although not examinable directly.

This method is the principal component or factor analysis (Sváb, 1979).

## 1. Materials and methods

Six wheat cultivars grown at the Cereal Research Institute (Szeged, Hungary) formed the test material used in the experiments. These and the methods of analyses were described in other papers (PALLAGI-BÁNKFALVI, 1984; PALLAGI-BÁNKFALVI & ÖRSI, 1984).

Three groups of analyses were carried out on the flours the results of which are listed in Tables 1, 2 and 3.

Table 1

*Tested properties of bread*

| Serial No. | Method used | Property studied |
|---|---|---|
| 1 | Measurement of volume | Volume |
| 2 | Planimetry | Surface of the cut |
| 3 | Calculated value | Form quotient |

Table 2

*Protein fractions of the flours studied*

| Serial No. | Fractionation procedure | Name of protein fractions |
|---|---|---|
| 1 | Fractionation according to Osborne | Salt-soluble protein |
| 2 | | Gliadin |
| 3 | | Glutenin |
| 4 | Fractionation with AUC[a] solution | AUC-soluble protein |
| 5 | | Gliadin dissolved in AUC |
| 6 | | AUC-insoluble fraction, soluble in alkali |
| 7 | Fractionation with acetic acid[b] | Acetic acid-soluble protein |
| 8 | | Acetic acid-insoluble gliadin |
| 9 | | Acetic acid-insoluble fraction, soluble in alkali |

[a] AUC = 0.1 *M* acetic acid + 3 *M* urea + 0.01 *M* cetyl trimethyl ammonium bromide

[b] Aqueous solution of 0.1 *M* acetic acid

Test loafs were baked from the flour samples according to the specifications of HUNGARIAN STANDARD (1971) and their volume, form quotient and the surface of the cut were determined.

To characterize the protein content of a flour three methods of fractionation were applied and the quantities of the fractions were determined by the modified biuret reaction.

Table 3

*Properties of the dough and gluten studied*

| Serial No. | Method of analysis | Property analysed |
|---|---|---|
| 1 | Kjeldahl's method | Raw protein content |
| 2 | Washed gluten | Quantity of wet gluten |
| 3 | | Quantity of dry gluten |
| 4 | | Spread |
| 5 | Valorigraph test | Water binding capacity |
| 6 | | Period of dough formation |
| 7 | | Period of stability |
| 8 | | Extent of softening |

The methods of fractionation were published previously (PALLAGI-BÁNKFALVI & ÖRSI, 1984).

The main protein fractions as shown in Table 2, (Osborne glutenin, AUC-soluble and insoluble fractions and acetic acid-soluble and insoluble fractions) were analyzed by SDS-PAGE after reduction with mercapto-ethanol. The gel was stained with Coomassie Brillant Blue R-250, washed with the methanol–acetic acid–water system and finally analyzed with densitometer (PALLAGI-BÁNKFALVI & ÖRSI, 1984). The molecular masses of the protein zones were established on the basis of the calibration curves obtained with the standard proteins applied simultaneously with the protein samples analyzed. The quantity of individual fractions was determined from the height of peaks in the densitogram and expressed as per cent of total protein. In the molecular mass range of 12 500 to 158 000 45 zones were distinguished and their quantity was established.

In order to be able to evaluate the data the molecular mass range was devided into 4 zones. Table 4 shows the quantitative distribution of varieties and protein fractions in the 4 zones. Except for one range they all contained at least 1 zone for each wheat variety (Table 4).

The mathematical statistical evaluation of results was carried out in 3 steps on the R-32 computer of the University Computer Center by the multi-variable method developed at the Department of Biochemistry and Food Technology, Technical University, Budapest. In the first step correlations within the variables tested were investigated in order to clarify relationships between variables.

By the use of the correlation matrix factor analysis was carried out and the eigen-values and eigen-vectors were determined. The communalities were estimated by the linear multi-variable correlation coefficient. New communalities were calculated with the help of the eigen-vector matrix. The

Table 4

*Percentage distribution of protein fractions between the molecular mass ranges chosen*

| Protein fraction | Wheat cultivar | Molecular mass range ($1 \times 10^3$) | | | |
|---|---|---|---|---|---|
| | | 12.5–33 | 34–45 | 46–72 | 74–158 |
| Protein fraction | Yubileinaya 50 | 26.95 | 29.45 | 21.56 | 22.20 |
| | GK-F2 | 25.09 | 25.69 | 17.82 | 31.26 |
| | Rana 1 | 4.00 | 45.95 | 33.65 | 18.02 |
| | Sava | 18.01 | 18.60 | 26.71 | 26.65 |
| | Partizanka | 23.51 | 18.45 | 28.14 | 29.97 |
| | GK-Basa | 7.47 | 24.31 | 9.30 | 50.70 |
| AUC-soluble | Yubileinaya 50 | 14.67 | 18.38 | 36.67 | 30.23 |
| | GK-F2 | 24.30 | 37.55 | 13.64 | 24.21 |
| | Rana 1 | 10.10 | 47.52 | 13.02 | 29.31 |
| | Sava | 34.19 | 33.05 | 22.51 | 10.54 |
| | Partizanka | 3.25 | 46.60 | 15.53 | 29.20 |
| | GK-Basa | 12.00 | 25.24 | 37.40 | 29.80 |
| AUC-insoluble | Yubileinaya 50 | 21.32 | 21.57 | 34.34 | 22.70 |
| | GK-F2 | 15.01 | 36.17 | 14.85 | 33.95 |
| | Rana 1 | 1.53 | 57.58 | 23.31 | 18.52 |
| | Sava | 5.79 | 53.42 | 15.49 | 25.27 |
| | Partizanka | 15.70 | 36.51 | 14.88 | 32.87 |
| | GK-Basa | 12.13 | 27.39 | 31.92 | 25.84 |
| Acetic acid-soluble | Yubileinaya 50 | 32.15 | 40.96 | 8.88 | 17.95 |
| | GK-F2 | 41.49 | 26.52 | 11.90 | 20.01 |
| | Rana 1 | 2.30 | 48.10 | 37.24 | 12.00 |
| | Sava | 33.66 | 57.40 | 5.71 | 3.18 |
| | Partizanka | 24.38 | 48.43 | 12.43 | 14.61 |
| | GK-Basa | 18.50 | 36.36 | 22.19 | 19.82 |
| Acetic acid-insoluble | Yubileinaya | 11.00 | 20.75 | 14.40 | 52.00 |
| | GK-F2 | 10.94 | 37.01 | 12.47 | 39.52 |
| | Rana 1 | 13.91 | 41.37 | 24.31 | 20.35 |
| | Sava | 0.00 | 20.68 | 32.02 | 47.16 |
| | Partizanka | 10.73 | 28.33 | 8.36 | 30.36 |
| | GK-Basa | 28.82 | 17.20 | 33.02 | 18.96 |

number of factors was established on the basis of relation $\lambda_i < 0.05$ n, where $\lambda_i$ = eigen-value, n = number of variables. The factor weight matrix describing individual variables was turned to the perpendicular axes by VARIMAX rotation.

In the knowledge of the factor weight matrix and the communalities factors were estimated by Bartlett's method using the following equation (JOHN & VAHLE, 1974):

$$F = Z\ B\ A(A^*\ B\ A)^{-1}$$

where $A$ and $A^*$ = factor weight matrix and its transposition,
$B$ matrix = diagonal matrix containing the reciprocals of the squares of communalities,
$Z$ = original standardized variables,
$-1$ exponent = stands for inversing the matrix in brackets; multiplications mark vectorial multiplications.

## 2. Results

Factor weight matrices obtained as the results of factor analysis of variable groups (bread properties, quantity of protein fractions, glutenin and dough characteristics, quantities of subfactors in molecular mass ranges of 158 000–74 000, 46 000–72 000, 35 000–45 000 and 12 500–33 000 resp., by SDS-PAGE) are shown in Table 5 to 11.
Communalities are also shown in all of the Tables being the measure of the ratio of variances of variables in the factors. The percentage of variance of the correlation matrix described by the factors is presented after each Table. Factor weights permit of estimating the participation of the weight of standardized variables in the factors.

Table 5

*Factor patterns of bread properties*

| Serial No. | Property | Factor weight (%) | | Communality |
|---|---|---|---|---|
| | | I | II | |
| 1 | Form quotient | −22 | 73 | 59 |
| 2 | Volume | 84 | −21 | 75 |
| 3 | Cut surface | 73 | −55 | 84 |

The two factors describe 73% of the variance of the correlation matrix

Table 6

*Factor patterns of the protein fractions*

| Serial No. | Property | Factor weight (%) | | | Communality (%) |
|---|---|---|---|---|---|
| | | I | II | III | |
| 1 | Salt-soluble protein | −74 | 55 | 4 | 83 |
| 2 | Osborne gliadin | −81 | 31 | 42 | 93 |
| 3 | Osborne glutenin | 85 | −40 | −28 | 95 |
| 4 | AUC-soluble protein | −39 | 86 | 32 | 99 |
| 5 | AUC-soluble gliadin | −40 | 75 | 50 | 98 |
| 6 | AUC-insoluble | 43 | −85 | −30 | 99 |
| 7 | Acetic acid-soluble | −66 | 62 | 41 | 99 |
| 8 | Acetic acid-insoluble gliadin | −28 | 35 | 90 | 99 |
| 9 | Acetic acid-insoluble glutenin | 75 | −34 | −51 | 93 |

The three factors describe 96% of the variance of the correlation matrix

Table 7

*Factor patterns of the gluten and paste properties*

| Serial No. | Property | Factor weight (%) | | Communality (%) |
|---|---|---|---|---|
| | | I | II | |
| 1 | Raw protein | 92 | 42 | 99 |
| 2 | Wet gluten | 78 | 52 | 88 |
| 3 | Dry gluten | 75 | 66 | 99 |
| 4 | Spread | 17 | 99 | 99 |
| 5 | Water absorption capacity | 74 | 68 | 99 |
| 6 | Period of pasta formation | 54 | 73 | 82 |
| 7 | Period of stability | −62 | 9 | 39 |
| 8 | Extent of softening | 55 | 51 | 57 |

The two factors describe 84% of the variance of the correlation matrix

Table 8

*Factor patterns in the molecular mass range of 158 000–74 000*

| Serial No. | Molecular mass ($1 \times 10^3$) | Factor weight (%) | | | | | Communality (%) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | |
| 1 | 158 | −6 | −6 | 0.3 | 4 | −17 | 4 |
| 2 | 125–130 | 96 | −1 | −1 | 1 | 0.4 | 92 |
| 3 | 117 | 99 | −2 | 6 | 10 | −3 | 99 |
| 4 | 100–97.5 | −17 | −76 | 43 | −32 | 18 | 93 |
| 5 | 95–96 | −16 | 95 | 24 | −14 | 6 | 99 |
| 6 | 86 | 63 | 10 | −64 | −38 | 18 | 99 |
| 7 | 85 | −8 | −13 | 76 | 9 | 4 | 62 |
| 8 | 81–82 | 16 | 1 | 12 | 61 | −9 | 42 |
| 9 | 78 | −11 | 15 | −37 | 5 | −1 | 17 |
| 10 | 76 | 36 | −1 | 35 | −57 | −40 | 74 |
| 11 | 74 | −15 | −27 | 7 | 8 | 77 | 70 |

The five factors describe 68% of the variance of the correlation matrix

Table 9

*Factor patterns of the molecular mass range of 72 000–46 000*

| Serial No. | Molecular mass ($1 \times 10^3$) | Factor weight (%) | | | | | Communality (%) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | |
| 1 | 72 | 16 | −72 | −24 | −15 | −19 | 70 |
| 2 | 68–69 | −22 | −48 | 10 | 42 | −10 | 48 |
| 3 | 66 | 3 | 27 | −61 | −14 | 39 | 62 |
| 4 | 65 | 12 | 22 | 81 | 10 | −5 | 72 |
| 5 | 62–63 | −41 | −13 | −16 | −55 | −29 | 60 |
| 6 | 60 | 83 | −8 | −30 | 0.5 | 46 | 99 |
| 7 | 57–58 | −23 | 17 | 5 | 72 | −12 | 61 |
| 8 | 55 | −13 | −73 | 12 | −24 | 15 | 65 |
| 9 | 53–54 | 59 | 11 | 28 | −16 | −14 | 49 |
| 10 | 50 | 8 | 2 | −16 | −2 | 84 | 74 |
| 11 | 46–47 | 7 | 28 | −38 | 35 | 5 | 36 |

The five factors describe 63% of the variance of the original correlation matrix

Table 10

*Factor patterns of the molecular mass range of 45 000–34 000*

| Serial No. | Molecular mass ($1 \times 10^3$) | Factor weight (%) | | | | | Communality (%) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | |
| 1 | 45 | 99 | 6 | 8 | −24 | 8 | 99 |
| 2 | 43–44 | −40 | −32 | −27 | −30 | −3 | 42 |
| 3 | 42–43 | −5 | 23 | −4 | 53 | −11 | 35 |
| 4 | 40 | −24 | −53 | −9 | −20 | 5 | 39 |
| 5 | 39 | −1 | 28 | 33 | 33 | −43 | 48 |
| 6 | 38 | 6 | 3 | −6 | −69 | −11 | 49 |
| 7 | 37 | 4 | 0 | −6 | 8 | 67 | 46 |
| 8 | 36 | 18 | 1 | 56 | 19 | 2 | 38 |
| 9 | 35.5 | 5 | 14 | −54 | 21 | 24 | 42 |
| 10 | 34.5 | 19 | 47 | 52 | −46 | 54 | 99 |
| 11 | 34 | 4 | 87 | 18 | −1 | −2 | 79 |

The five factors describe 57% of the variance of the original correlation matrix

Table 11

*Factor patterns of the molecular mass range of 33 000–12 500*

| Serial No. | Molecular mass ($1 \times 10^3$) | Factor weight (%) | | | | | Communality (%) |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | |
| 1 | 33 | −49 | 62 | −6 | −25 | −3 | 69 |
| 2 | 32 | −10 | −72 | 25 | 2 | −5 | 60 |
| 3 | 30 | −10 | 4 | −17 | 67 | −3 | 49 |
| 4 | 29 | 15 | −1 | 11 | 60 | −3 | 39 |
| 5 | 28 | −11 | −60 | −21 | −31 | 18 | 54 |
| 6 | 27 | 29 | 9 | 48 | −2 | −2 | 32 |
| 7 | 25–26 | −73 | 0.3 | −16 | 34 | 13 | 69 |
| 8 | 22–23 | 12 | −7 | 99 | 3 | 1 | 99 |
| 9 | 20–19 | −82 | −2 | −5 | 5 | −17 | 70 |
| 10 | 17–18 | −4 | −9 | 48 | −2 | −7 | 25 |
| 11 | 14–15 | −80 | −6 | −12 | 14 | 48 | 91 |
| 12 | 12.5 | −5 | −16 | −8 | −10 | 98 | 99 |

The five factors describe 63% of the variance of the original correlation matrix

Using the factor weight matrix and the communalities coefficients of the equation for the estimation of factors were calculated. The original data were substituted into the equation and used to calculate the estimated value of the factors. Finally instead of the original correlations of the 65 variables only the correlations of 27 factors had to be examined by correlation calculations. The correlation coefficients of factors of the same group were not necessary to calculate since they are equal to 0. Thus, instead of $26 \times 27 = 702$ correlation coefficients only 312 had to be calculated and analyzed and in addition most of the disturbing correlations were eliminated. In Table 12 only the significant correlation coefficients were presented.

Table 12

*Significant correlation coefficients of the factors at the probability level of 95%*
(The hundred-fold of the correlation coefficients is given)

| Factor group | Factor | Bread | | Protein fractions | | | Gluten and pasta | |
|---|---|---|---|---|---|---|---|---|
| | | I | II | I | II | III | I | II |
| Acetic acid- soluble 12 500–33 000 | II | −94 | 94 | — | — | — | — | — |
| | III | — | — | — | — | — | 94 | 95 |
| | IV | −88 | 89 | — | — | — | — | — |
| | V | 92 | −93 | — | — | — | — | — |
| AUC-soluble 45 000–74 000 | I | — | — | −88 | 88 | 88 | — | 83 |
| | II | — | — | 88 | −88 | −88 | — | −83 |
| | III | — | — | 88 | −88 | −88 | — | −83 |
| | IV | — | — | 88 | −88 | −88 | — | −83 |
| | V | — | — | −88 | 88 | 88 | — | 83 |
| AUC-soluble 74 000–158 000 | I | — | — | −86 | 86 | 85 | 91 | 92 |
| | II | — | — | 84 | −84 | −86 | — | — |
| | III | — | — | 81 | — | −83 | — | — |
| | IV | — | — | −91 | 90 | 91 | — | — |
| | V | — | — | 89 | −88 | −90 | — | — |
| AUC-insoluble 12 500–33 000 | IV | −90 | 91 | — | — | — | — | — |
| | V | 85 | −86 | — | — | — | — | — |
| AUC-insoluble 45 000–74 000 | I | — | — | — | — | — | 92 | 92 |
| | II | — | — | — | — | — | −92 | −92 |
| | III | — | — | — | — | — | −92 | −92 |
| | IV | — | — | — | — | — | −92 | −92 |
| | V | — | — | — | — | — | 92 | 92 |
| AUC-insoluble 74 000–158 000 | I | — | — | −92 | 92 | 93 | — | — |
| | II | — | — | 91 | 91 | −92 | — | — |
| | IV | — | — | −91 | 91 | 92 | — | — |
| | V | — | — | 91 | −91 | −92 | — | — |
| Osborne glutenin 12 500–33 000 | I | — | — | −89 | 89 | 91 | — | — |
| | II | — | — | −82 | 81 | 83 | — | — |
| | III | — | — | −89 | 88 | 90 | — | — |
| | IV | — | — | −87 | 87 | 88 | — | — |
| | V | — | — | 86 | 85 | 97 | — | — |
| Osborne glutenin 34 000–45 000 | I | — | — | — | — | — | — | −83 |
| Osborne glutenin 46 000–72 000 | I | — | — | — | — | — | 99 | 99 |
| | IV | — | — | — | — | — | 96 | 97 |
| | V | — | — | — | — | — | −87 | −87 |

It can be established from the correlation coefficients that the quantities of the protein fractions in flour are determined by the AUC-soluble fractions in the molecular mass range of 45 000–158 000, the AUC-insoluble fractions in the molecular mass range of 74 000–158 000 and the glutenin fractions

according to Osborne in the molecular mass range of 12 500–33 000. As it can be seen in Table 4 these constitute 27–67%, 18–39% and 0–11%, respectively, of the total amount of protein.

Properties of the gluten and of dough are determined by the acetic acid-soluble fraction in the molecular mass range of 12 500–33 000, the AUC-soluble fraction in the molecular mass range of 75 000–158 000, the AUC-insoluble fraction in the molecular mass range of 45 000–75 000. As it is shown in Table 4 these amount to 2–34%, 27–67% and 15–34%, respectively, of the total protein content.

Properties of bread are determined by the acetic acid-soluble fraction in the molecular mass range of 12 500–33 000 and the AUC-insoluble fraction in the molecular mass range of 12 500–33 000. According to Table 4 these constitute 2–34% and 1.5–22%, respectively, of the total amount of protein.

Results have proven the expectations and very highly significant correlations were found.

To study the correlations in the produce of several years and other wheat varieties further investigations are intended.

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# Abstracts

of papers presented at the

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THE MICROBIOLOGICAL SECTION AND FOOD ANALYTICAL EXPERT COMMITTEE OF THE HUNGARIAN SCIENTIFIC SOCIETY FOR FOOD INDUSTRY
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## PRODUCTION OF ENZYME PREPARATIONS FOR THE FOOD INDUSTRY

P. Biacs

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The two main fields of research and development in biotechnology with the highest prospects of utilization are:

— enzyme technologies or the development of enzyme engineering, that is utilization of isolated and immobilized biological units or enzymes in food production;
— genetic engineering permitting of translation of genetic information among plants, animals and mircoorganisms thereby producing species with new properties.

There are five main fields of application available for Hungarian experts of biotechnology where traditional trends in fermentation research and development may be enriched through genetic engineering and enzyme technology:

— new plant species in agriculture and food production and new propagation techniques in animal husbandry;
— complex, energy and material saving processing of the biomass formed in the food industries, with enzymes and starter cultures;
— development of new sources of energy (biogas, bioalcohol) by more effective biological and technical means;
— biological techniques applicable in environmental protection to reduce pollution of the environment (waste water treatment, nitrogen fixing bacteria replacing synthetic fertilizers);

— sanitary techniques ensuring better hygienic conditions and combatting endemic diseases (vaccines, enzyme diagnostics).

From the point of view of the food industry the development of enzyme technologies is of particular importance. These permit more careful treatment of raw materials of plant and animal origin, protect their biological values or produce new values in the course of their processing.

The production of enzyme preparations could hardly keep abreast with the increasing demand in Hungary. The many-sided requirements necessitate a definite step forward in the manufacture and product development and in the production of high activity and stable enzyme preparations (powdered or granulated). This task requires the thorough knowledge of the tissue and cellular enzymes in different raw materials (plants, animals, microorganisms), the survey of genetic information and its complementation by gen transfer as well as a high grade enzyme manufacturing organization and management and the careful testing of the final product. The program of the 4th Conference on Enzymology includes all these fields and I am pleased to state that thereby the appropriate expert background and erudition is ensured for the realization of the program as laid down for the near future.

With the cooperation of chemists, biologists and engineers a new branch of science and of industry is in the course of development in Hungary: enzyme engineering among the aims of which particular importance is attached to the design and operation of bioreactors, the immobilization of biocatalysts and the conscious adaptation of enzyme activity by means of physical methods (adsorption, covalent binding). The future tasks of food economy stress the complex and combined application of enzymes (cellulase, pectinase) and of enzyme systems coupled naturally and artificially. Both ways are expensive and it requires a great number of innovative steps to achieve the best possible solutions under the conditions of this country.

One of the aims of this Conference is to reduce the dispersal highly perceptible at present and provide a forum for the mutual exchange of technical experiences and permit of coordination indicated and necessary as yet. In the knowledge of the results achieved so far it is our duty to promote the realization of research programs and projects the aim of which is the modernisation and propagation of enzyme manufacture and application.

# ENZYME INHIBITORS IN CEREALS

R. LÁSZTITY

*Department of Biochemistry and Food Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

Researchers and practical experts became acquainted with the problem of natural enzyme inhibitors in relation to soya. Intensive research led to the knowledge of the trypsin inhibitors of soya on a molecular level. The Japanese researchers ODANI and IKENAKA (1973) determined the amino acid sequence of the Bowman–Birk inhibitor and in the same year two researchers KOIDE and IKENAKA (1973) discovered the primary structure of the Kunitz inhibitor. Trypsin inhibitors of cereals (wheat germ) were first isolated in 1969 by HOCHSTRASSER and WERLE (1969). Later other researchers also succeded in detecting proteins of trypsin inhibitor action. It is interesting that the endosperm contains trypsin inhibitors, too. The investigations have shown the trypsin inhibitors to be proteins of low molecular mass (10–20 kilodalton) mostly of alkaline character.

The trypsin and chymotrypsin inhibitors in rye and triticale were first described in 1974 (TSEN, 1974). The extreme thermostability of the trypsin inhibitor was stressed and a non-competitive action mechanism was assumed. Gel-electrophoretic investigations proved the heterogeneity of the inhibitors.

The amylase inhibitors in wheats were first mentioned by KNEEN and SANDSTEDT (1943). However, more detailed studies were launched only in 1970, when SHAINKIN and BIRK (1970), applying modern methods of protein analysis, succeeded in separating relatively pure alpha-amylase inhibitors and studied their specificity. It was shown that inhibitors of the lowest molecular mass inhibit primarily the amlyases in insects. Among the inhibitors of medium molecular mass some inhibit the amylases of saliva and chickens, while amylases of cereals are inaffected.

In relation to enzyme inhibitors thionins should also be mentioned. These are proteins of low molecular mass (6–8 kilodalton), contain a high amount of basic amino acid and cysteine (cystine) and are found in every kind of cereals. The primary structure of most thionins is known. Their comparative analysis was carried out at the Department of Biochemistry and Food Technology, Technical University, Budapest (BÉKÉS & LÁSZTITY, 1981a, b). Their biological role and their biochemical mechanism of action has not been clarified yet. Their amlyase inhibiting effect has been proven lately.

From the point of view of practice (nutrition, animal feeding) inhibitors, on the one hand, interfere with the digestibility of proteins or carbohydrates. On the other, methods are sought to inhibit them, if necessary. The trypsin inhibitors in soya have been thoroughly investigated and their inactivation was

found to be necessary. Reliable data on the trypsin (chymotrypsin) inhibitor in grain are not available. These inhibitors are of lower activity and thus their inactivation is considered not to be necessary. The inhibitory effect of alpha-amylase inhibitors can be measured in men and animals, particularly upon consumption of native starch. In the case of digested starch the inhibitory effect is negligible.

The simplest and most economic way of inactivation is heat treatment.

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# BIOCHEMICAL CHANGES IN EDIBLE MUSHROOMS DURING STORAGE

D. Törley and E. Györey-Vadon

*Department of Biochemistry and Food Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

Biochemical changes of differently developed fruiting bodies of the cultivated mushroom *(Agaricus bisporus)* were studied, during storage, by polyacrylamide gel electrophoresis. Immature, closed fruiting bodies were collected for the first group, mushrooms with closed pileus were purchased on the market for the second, and mushrooms with open pileus formed the third group. Conditions of storage were the following:

7 days at room temperature, without air-conditioning,
7 days at room temperature and 72% relative humidity,
9 days in the refrigerator at 5–7 °C and 98% relative humidity,
9 days in frozen state at −18 °C and 98% relative humidity.

The protein patterns and the isoenzymes of some dehydrogenases and polyphenoloxydase were determined from the cell-sap, three parallel samples were run in all cases. The pherograms were evaluated by densitometry.

In general, it can be stated that during biological ripening the number of the isoenzymes increases, and the same is valid for the storage period. In young, closed fruiting bodies three isoenzymes each of lactate dehydrogenase, mannitol dehydrogenase, succinate dehydrogenase, malate dehydrogenase and polyphenoloxydase were found, and during storage the number of the dehydrogenases increased to 4. The enzyme activities increased threefold. In mushrooms with open pileus 4 polyphenoloxydase and 5 dehydrogenase isoenzymes could be detected; during storage the number of the dehydrogenase isoenzymes was doubled, polyphenoloxydase did not change, and all enzyme activities were doubled.

The majority of the newly formed isoenzymes appeared in the fraction of higher mobility, probably as the result of catabolic processes. In the protein patterns 16–22 bands could be detected, and this number increased during storage to 22—27. Cold storage proved to be the best as the slightest changes in proteins occurred under those conditions. The number of the isoenzymes depends principally on the stage of development or maturation and is not influenced by storage conditions.

## CHANGES IN THE MALATE DEHYDROGENASE (EC 1.1.1.37) ACTIVITY AND MALIC ACID CONTENT IN GREEN PAPRIKA DURING FROZEN STORAGE

E. Bogdán-Molnár, K. Gasztonyi and E. Epinger

*Departmental Group for Food Chemistry, University of Horticulture, H-1118 Budapest, Somlói út 14–16. Hungary*

Changes in malate dehydrogenase (MDH) activity and malic acid content in green paprika were investigated in an earlier study during storage between +4 and +22 °C and both malic acid content and malate dehydrogenase activity were found to decrease linearly during 11 to 24 days storage. The value of $Q_{10}$ was about 2. In the present study the investigation was extended over the temperature range below freezing point. The experiments were carried out with three paprika cultivars: Soroksári, Bogyiszlói and Fehérözön. The washed paprika was frozen at −25 °C (for 30–60 min) and stored at −22 °C for a maximum of 12 months. The paprika was prepared for sampling in three different ways:

– till sampling the paprika was kept in frozen storage and it was prepared for analysis in the frozen state (experiment *A*);

— 12 h prior to sampling it was removed from frozen storage, thawed and then prepared for analysis (experiment *B*);
— paprika was thawed for 12 h then refrozen and kept in frozen storage for 2–60 days prior to sampling (experiment *C*).

The aim of experiment *C* was to model a possible rupture in the refrigerated chain.

Malic acid content and malate dehydrogenase activity determination were based on the following reversible reaction:

$$\text{L-malate} + \text{NAD}^+ \xrightleftharpoons{\text{L-MDH}} \text{oxaloacetate} + \text{NADH} + \text{H}^+$$

Changes in NADH concentration were measured with a spectrophotometer at 340 nm.

The following results were obtained:

Samples *A*, stored for 3–12 months had an average malic acid content of $7.9 \pm 0.8$ mmol $g^{-1}$. A similar value was obtained for samples *B*, studied in the thawed state: $8.4 \pm 1.2$ mmol $g^{-1}$. The malic acid content in samples *C*, thawed then refrozen, was found to be $8.7 \pm 0.7$ mmol $g^{-1}$. Thus the malic acid content of the three samples treated in three different ways did not show significant difference.

The average enzyme activity in sample series *A* was found to be $2.73 \pm 0.6$ mmol $g^{-1}$; in sample series *B* a similar result was obtained: $2.70 \pm 0.5$ mmol $g^{-1}$ while in series *C* a value about half of the above was found: $1.02 \pm 0.3$ mmol $g^{-1}$. The results show that thawing followed by refreezing and further refrigerated storage significantly reduced the enzyme activity.

To evaluate the results the specific enzyme activities were calculated showing the MDH activity per unit malic acid molecule. The quotients belonging to the three experimental series are as follows: series *A*: $345 \times 10^{-3}$, series *B*: $321 \times 10^{-3}$ and series *C*: $117 \times 10^{-3}$. In mature samples not stored, the enzyme activity was 0.60 mmol $g^{-1}$, the malic acid content 1.7 mmol $g^{-1}$ and the specific activity $352 \times 10^{-3}$.

## CHANGES IN THE PEROXIDASE ACTIVITY OF QUICK-FROZEN BROCCOLI DURING PROCESSING AND STORAGE

A. Kampis[a], O. Bartucz-Kovács[b], L. Vámos-Vigyázó[a] and Á. Hoschke[a]

[a]*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
[b]*Hungarian Deep Freezing Industry, H-1054 Budapest, Dorottya u. 1. Hungary*

Incidental brown discolouration was found to occur in the thick stem parts of quick-frozen broccoli. The phenomenon was assumed to be due to insufficient blanching that manifested itself in a high residual peroxidase (POD) activity.

In order to clarify the possible relationship between POD activity and the browning of broccoli, the activity changes in the soluble and the ionically cell-bound enzyme fractions were followed during processing and frozen storage (6 months, −18 °C).

The enzyme was separately extracted from the stem and the floret parts by 2-min homogenization of the comminuted vegetable in an appropriate buffer. 0.02 mol $dm^{-3}$, pH 6.0 acetate buffer was used to obtain the soluble, and the same buffer containing 0.8 mol $dm^{-3}$ $CaCl_2 \cdot H_2O$ to extract the ionically bound fraction. Activity was assayed at pH 6.0 by a modification of the procedure described by MIHÁLYI and VÁMOS-VIGYÁZÓ (1975) and related to solids content. The reproducibility of the method as expressed by the variation coefficient was, for extracts prepared in parallel, below ± 10%.

Four-min blanching at 95 °C decreased POD activity in the stem parts by one, in the floret parts by two orders of magnitude, whereby the soluble fraction seemed somewhat more resistant. A further decrease in activity of about 50% occurred during freezing.

During frozen storage soluble and cell-bound activities varied in different ways in the stem and floret parts. Ionically bound activity decreased in both parts throughout storage. The changes were most marked during the first and the first two months, respectively. At the end of the 6-month period residual activities in stem and floret amounted to 49% and 16%, respectively, of the values found directly after freezing. In the floret parts the course of changes in soluble activity was similar to that observed in the stem. In 6 months, activity was reduced to 32% of the value measured directly after freezing. In the stem, soluble activity proved much more stable. A loss of less than 10% occurred mainly during the first month.

At the end of the storage period total POD activity in the stem was about 18 times that found in the floret, and about 75% of it was soluble. Further research will be focused on this fraction.

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# COMPARISON OF SOME ENZYME ACTIVITIES IN *TRITICUM AESTIVUM* AND *TRITICUM DURUM*

A. Párkány-Gyárfás, L. Vámos-Vigyázó and Á. Hoschke

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The activities of some enzymes of importance in the processing technologies of *T. aestivum* (*Ae*) and *T. durum* (*D*) were followed from flowering till harvest in samples of one variety each (Martonvásári 4 and Basa), grown on experimental parcels in the same year (1982) but at different locations. Samples were taken weekly during the 6-week ripening period. Sampling of *D* was performed always one week later than that of *Ae*. Activities were determined in water extracts of wheat kernels. The essay methods for amylase, peptide hydrolase, peroxidase and lipoxygenase had been described in detail elsewhere (Vámos-Vigyázó, 1984), that of polyphenol oxidase was adapted from the literature (Lamkin et al., 1981). All the activities were expressed in arbitrary units (U): $1 = 10^{-3} \Delta$ A min $^{-1}$ (A = absorbance). Activity values were related to the mass of the kernel. Amylase isoenzyme patterns in water extracts of resting wheat kernels (8–10 months after harvest) were compared in basic (pH 7.9) and acidic (pH 4.8) polyacrylamide disc-gel-electrophoretic systems (PAGE) (Davis, 1964; Reisfeld et al., 1962).

At the first sampling, i.e., 4 to 7 days after flowering, the activities of all the enzymes were higher in *D*. In both *Ae* and *D* amylase activity was highest at the first sampling, the decrease that followed till the end of the fourth week was more expressed in *D*. In the last two weeks of ripening, activity somewhat rose in *Ae*, while it remained practically constant in *D*. Peptide hydrolase activity first increased up to the fourth and second sampling in *Ae* and *D*, respectively, whereafter a sudden drop — again more pronounced in *D* — was observed till the sixth and fifth samplings. In the last period of ripening activity variations were but slight. Peroxidase activities rose steeply in both samples during the first four weeks. The value reached by that time was maintained in *Ae* till harvest, while a sudden drop to about the initial value occurred in sample *D*. Lipoxygenase varied in *Ae* and *D* in a very similar way: its low initial value did not change much during the first 14 and 7 days, respectively, whereafter a sudden rise till harvest could be observed in both samples. Finally, polyphenol oxidase activity increased in *Ae* during the whole period of ripening more or less according to a sigmoidal curve, while in *D* a sudden rise up to the second sampling was followed by a 3-week period of decrease to show a somewhat less steep increase during the last week of ripening.

As a result of all these changes the activities of all the five enzymes investigated were, at harvest, lower in *D*. The respective values for *Ae* and *D* were 10 $\pm$0.2 U and 2.2 $\pm$ 0.04 U for amylase, 28 $\pm$ 0.6 U and 19 $\pm$ 0.4 U for

peptide hydrolase, 8.5 ± 0.2 kU and 6.2 ± 0.3 kU for peroxidase, 3.3 ± 0.2 kU and 1.2 ± 0.06 kU for lipoxygenase and 25 ± 1 U and 14 ± 0.5 U for polyphenol oxidase (1 kU = 1000 U).

PAGE revealed both α and β-amylase isoenzymes of *Ae* to migrate in the basic system towards the anode, while only α-amylases could be detected in the same system in *D*. The β-amylase fractions of *D* migrated in the acidic system towards the cathode.

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# INVESTIGATIONS INTO THE POLYPHENOL OXIDASE ACTIVITY OF DURUM WHEATS

J. TEMESVÁRI

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Brown discolouration of pasta produced from durum wheat is attributed to the action of polyphenol oxidase (PPO). In order to investigate PPO activity in durum wheat varieties from different locations in Hungary as well as the changes in activity occurring during processing, a method was adapted from the literature (LAMKIN et al., 1981).

Ground sample of durum wheat, grit, flour and pasta were comminuted where necessary, mixed with water in appropriate ratios, shaken for 1 h on a laboratory shaker, centrifuged and filtered. Activity was determined in dilutions of the filtrate (enzyme extract). Out of five substrates, the highest reaction rate could be obtained with DL-3,4-dihydroxyphenylalanine (L-DOPA) at a concentration of 2.5 mmol $dm^{-3}$. (Higher concentrations inhibited the reaction.) Optimum pH and temperature were 8.3 and 37 °C, thus these parameters were adapted for kinetic spectrophotometric measurements at 430 nm. Activity was calculated, by regression analysis, from the linear section of the absorption vs. reaction time plot related to 1 g of durum wheat or product solids. Unit activity was arbitrarily selected as $10^{-3}$ $\Delta A$ $min^{-1}$ (A = absorbance). In addition, zymograms of the extracts of two wheat varieties were prepared according to DAVIS (1964).

Activities varied considerably both with varieties and with locations. Differences between the samples were found, by analysis of variance, to be significant at the probability level of 0.1%. Low activities were found in the varieties GK Basa and GK Madur, medium values in Attila and high ones in GK Minaret and Miradur. Samples from the locations Iregszemcse and Jászboldogháza showed higher activities than those from Tordas, except for the spring wheats GK Miradur and Attila.

The zymograms of the cultivars GK Basa and GK Minaret showed 8 and 5 bands, respectively, on staining with L—DOPA. Only two of these had similar relative mobilities in the zymograms of the two cultivars (0.21–0.24 and 0.32–0.33, respectively).

The analysis of the semi and end products revealed the enzyme to be only partly destroyed during processing: 30% of the activity of wheat (GK Basa) was found in grits or flour and about 10% in pasta. This latter value ($5.0 \pm 0.2$ U $g^{-1}$), although slight, might contribute to discolouration. Commercial pasta samples analyzed showed similar residual activities.

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# CHANGES IN THE ACTIVITIES OF RESPIRATION ENZYMES IN APPLES DURING STORAGE

P. MERÉSZ,[a] R. LÁSZTITY,[a] P. MOHÁCSI,[b] P. SASS[a] and A. SUSITS[a]

[a]*Department for Biochemistry and Food Technology, Technical University, Budapest, H-1521 Budapest. Hungary.*

[b]*Department of Fruit Growing, University of Horticulture, H-1118 Budapest, Villányi út 35. Hungary*

One of the conditions of selling fruits as an important product of Hungarian agriculture more economically on foreign markets is to maintain their quality during more or less prolonged storage.

A factor highly affecting the quality of apples during storage is their intense respiration. The present study was aimed at the elucidation of the mechanism of respiration and of checking of its control points.

The dehydrogenase enzymes present in the apple tissue were investigated for their activity and for their effect on gross respiration intensity.

Four apple varieties were studied during two years: Jonathan, Idared, Goldenspur and Starcrimson. Jonathan apples were irradiated at the beginning of the storage period with radiation doses of 0.5, 1.0 and 1.5 kGy, respectively. These were then compared with apples kept in storage for the same time, but not given radiation treatment. No difference was observed in colouration, total acid content, sugar content, soluble solids content and aroma composition of the apples treated and non-treated. Pectinesterase activity in the irradiated apples was found substantially lower, however, the change occurring during storage did not differ from that in the untreated sample. Polygalacturonase activity was not affected by irradiation. Therefore it may be concluded that the better rheological quality of stored apples is the consequence of reduced pectinesterase activity. By the end of the storage period the PE activity increased, while the PG activity was reduced.

In the second part of this study the changes occurring during storage in the activities of dehydrogenase enzymes of different specificity were investigated. The dehydrogenases of different apple cultivars were found to differ substantially in their activities. While these are high in Jonathan, low values were obtained in Goldenspur. Mitochondria and cytoplasm were separated in the cortex. A high NAD specific malate dehydrogenase (MDH) activity was measured in both, but NADP specific activity could not be detected. Irradiation reduced the activities of both MDH and isocitrate dehydrogenase (ICDH) by 10–30%. Since during storage the activities of dehydrogenases were reduced only by 5–10%, it seems that the reduction in respiration intensity is due not so much to the reduction in dehydrogenase activity but rather to reduction in cell organisation .

## SEPARATION OF THE ISOENZYMES OF POLYPHENOL OXIDASE AND INVESTIGATIONS INTO THEIR SUBSTRATE SPECIFICITY

A. Cs. Pavisa,[a] J. Hámori-Szabó,[b] P. Sass[b] and L. Vámos-Vigyázó[a]

[a]*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
[b]*Department of Fruit Growing, University of Horticulture, H-1118 Budapest, Villányi út 35. Hungary*

Changes in polyphenol oxidase (PPO) activity, polyphenol content and rate of enzymatic browning as observed during ripening and storage of the three major apple cultivars (Jonathan, Starking, Golden Delicious) grown in Hungary (Vámos-Vigyázó et al., 1980) suggested PPO isoenzyme composition to have a bearing on browning tendency.

Soluble enzyme preparations were obtained from apples by extraction, with 0.1 mol $dm^{-3}$, pH 7.5 phosphate buffer, of acetone powders prepared according to Drawert & Görg (1972).

By applying acidic and basic polyacrylamide disc gel-electrophoretic systems (DAVIS, 1964; REISFELD et al., 1962) the isoelectric points of apple PPO isoenzymes were found to be in the range of pH 4.5–8.1. Three substrates: (−)-epicatechin, (+)-catechin and DL-dihydroxyphenylalanine (DOPA), (from Calbiochem, San Diego, California) yielded similar electrophoretic patterns, thus the first one was adopted for further work as this was found to be the principal browning substrate in all three cultivars (NÁDUDVARI-MÁRKUS & VÁMOS-VIGYÁZÓ, 1984). Chlorogenic acid, catechol and pyrogallol proved less suited for staining.

In the acidic system only one active band of low mobility was detected. The basic system proved more suited for differentiation between the cultivars. Starking extracts yielded three to four distinct fractions in the relative mobility ($R_f$) ranges of 0–10, 20–30, 40–50 and 60–70, respectively. The parts of the gel between the latter three fractions were also faintly stained. The fraction of the lowest mobility did not appear in any of the samples. The main fraction ($R_f$ = 40–50) was present in the extracts of all the cultivars. The pattern of Golden Delicious showed additional distinct bands of $R_f$ 20–30 and in some samples also of $R_f$ 60–70. The gel between the fractions was faintly stained. Only the main isoenzyme could be detected in the extract of Jonathan, this was surrounded by a faint diffuse zone.

The conclusions to be drawn from the results are as follows.

No relationship between substrate specificity as obtained in browning activity measurements and suitability for zymogram staining was found. The rate of browning of Jonathan and Starking proved much higher on chlorogenic acid (3–5 times) and catechol (3–7 times) than on the catechins or L—DOPA, while pyrogallol was a poor substrate for quantitative measurements, too (VÁMOS-VIGYÁZÓ & GAJZÁGÓ, 1978).

The differences in isoenzyme composition of the three cultivars were of quantitative rather than qualitative character. The number of active fractions parallelled the intital rate of browning and PPO activity of the samples.

Thus, the electrophoretic separation of isoenzymes might be used, similarly to PPO activity or browning measurements in selecting apples of slight browning capacity for processing purposes.

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# INACTIVATION OF ENZYMES IN FOOD PROCESSING BY HEAT TREATMENT

K. VUKOV

*Institute of Food Technology, University of Horticulture, H-1118 Budapest, Ménesi út 45. Hungary*

The most effective way of heat preservation is the total destruction of every life function by heat treatment: thermo-abiosis. One of the tasks involved is the irreversible inactivation of enzymes. Inactivation of enzymes in plant and animal tissues used as food is a direct consequence of the fact that the rate of cytoplasm destruction in tissue cells increases with increasing temperature according, in general, to the rate of a first order reaction. In most cases the effect of temperature on the rate constant of the reaction can be described with the Arrhenius equation:

$$k_r = k_0 \, e^{-\frac{E_a}{RT}}$$

where:

$k_r$ = actual reaction rate constant,
$k_0$ = reference reaction rate constant,
$e$ = base of natural logarithm,
$E_a$ = activation energy,
$R$ = universal gas constant,
$T$ = absolute temperature.

The equation is generally valid by the temperature ranges of 40–60 K, where the activation energy is constant. Within narrower temperature limits it is expedient to apply the simplified Arrhenius relationship as used in theoretical canning technology (HERMANN, 1976; VUKOV, 1982). In this relationship the logarithm of the inactivation period, i.e. the time required to reduce the initial activity to its one millionth part ($10^{-6}$ $A_0$) ($A_0$ = initial activity) is considered to be a function of temperature.

Thus, the relation of the inactivation period belonging to a given temperature, $t_T$, and the inactivation period belonging to the reference temperature $t_r$, is expressed as follows:

$$\frac{t_T}{t_r} = \frac{T - T_r}{z}$$

where

$T$ = temperature studied,
$T_r$ = reference temperature,
$z$ = temperature dependent characteristic.

The reference temperature of enzyme inactivation in the canning industry, $T_r = 100$ °C = 373.2 K. The time required at this temperature to inactivate the given enzyme is the enzyme inactivation number $E$.

By means of the above relationship the enzyme inactivation number $E$ and the temperature dependent charactristic $z$ can be calculated from the enzyme inactivation periods as measured at any two temperatures (VUKOV, 1982). In the knowledge of these values the enzyme inactivation equivalent, number $E'$, of the heat load given the food during heating at any rate, can be calculated. The enzyme becomes inactivated when the equivalent is higher than the inactivation number ($E' > E$).

The inactivation rate of enzymes, just as any protein denaturation rate, is highly dependent on the medium: on its water activity ($a_w$), pH, ionic strength, presence of certain ions, e.g. heavy metal ions. Isoenzymes become inactivated at various rates depending to different extents on the above factors. The temperature dependence of inactivation may also vary to a great extent as a function of the above factors.

Since from the point of view of preservation the most important enzymes are oxidases and among them peroxidases are of the highest heatresistance, most data found in the literature deal with the inactivation of peroxidases. Twenty-two data given by ten authors show that at the reference temperature $T_r = 100$ °C the enzyme inactivation time $E$ varies between 0.08 and 42 min. The lowest number was found for peaches, the highest for green peas. The value $z$ characterizing temperature dependence falls between 5.2 and 46.9 °C. The first value relates to oranges, the latter to snap beans. The most frequently occurring $z$ values are between 16 and 20 °C. The heat inactivation characteristics of catalase and phenoloxidase vary also within a broad range.

As regards the isoenzymes, some of them are highly thermostable and when heat-inactivated their activity is reversible. VÁMOS-VIGYÁZÓ and coworkers (1979) found kohlrabi to become inactivated at about 80–90 °C with a temperature dependence of $z = 10$ °C. However, during further heating inactivation slows down and stops completely when 2–2.5% of the initial activity is reached. This residual activity is maintained for a long time and during cooling subsequent to heat treatment may even slightly increase. In such cases the inactivation number $E$ cannot be interpreted.

In the knowledge of the above facts the conclusion can be drawn that the thermoresistance of enzymes in different materials is substantially different. Thus, in every case it is necessary to experimentally determine the kinetic

characteristics. An elegant way of doing this is to apply the method developed by REICHART (1983). Interpretation of the number $E$ and generally of the heat treatment equivalent, its calculation and a program for a small computer were developed by KÖRMENDY (1982).

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# KINETICS OF GROWTH AND PECTIN LYASE FORMATION OF *ASPERGILLUS NIGER*

NGUYEN XUAN THIEN and K. ZETELAKI-HORVÁTH

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The variant of an *Aspergillus niger* strain obtained by treatment with sodium azide (ZETELAKI-HORVÁTH et al. 1981) was grown in a 10 $dm^3$ glass fermentor. The nutrient medium contained natural pectin (beet extract) and was complemented with a nitrogen source and mineral salts. The effect of beet extract concentration on growth and pectin lyase formation was studied by the method of KONO and ASAI (1968).

In contrast to previous observations (ZETELAKI-HORVÁTH, 1978) the growth of the Aspergillus strain applied in the present experiments could not be subdivided into two growth phases, the growth curve consisted of four phases.

Growth rates ($k$) in the logarithmic phase of growth were reduced with increasing beet extract concentration while the maximal mycelium yields ($x_M$) increased. Growth periods required to reach maximum mycelium yield in beet extracts of 1, 2, 3 and 4% solids content were 36 and 48 h ($t_M$), respectively.

Pectin lyase formation of the culture is not a growth-related product formation. Enzyme synthesis is mainly occurring in resting cells as the product formation coefficient ($k_{p1}$) of growing cells was found to be 0, while that of the

resting cells ($k_{p2}$) and that of the final product formation ($k'_p$) gave positive values. Thus, the production of pectin lyase belongs to type III product formation according to KONO and ASAI (1968). The product formation coefficients ($k_{p2}$) of the resting cells showed a decreasing trend with increasing beet extract concentration. The yield constants of final product formation ($k'_p$), on the other hand, increased with increasing carbon source concentration. The highest pectin lyase concentration was achieved in a medium of 3% beet extract ($p_M = 4.24$ $\mu$mol min$^{-1}$ cm$^{-3}$), while the lowest pectin lyase formation (1.60) $\mu$mol min$^{-1}$ cm$^{-3}$) was observed in the medium containing 4% beet extract.

Applying beet extract of 1, 2, 3 and 4% solids content the cultivation period required to achieve maximum pectin lyase yield ($t_{Mp}$) was found to be 66 and 72 h, respectively.

The most important kinetic constants of growth and pectin lyase production of *Asp. niger* in media of different beet extract concentrations are as follows:

| Beet extract (%) | $t_M$ | $x_M$ | $k$ | $k_{p1}$ | $k_{p2}$ | $k'_p$ | $p_M$ | $t_{Mp}$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 36 | 4.3 | 0.150 | 0 | 0.1210 | 0.054 | 2.20 | 66 |
| 2 | 36 | 5.8 | 0.141 | 0 | 0.0133 | 0.069 | 3.30 | 66 |
| 3 | 36 | 8.3 | 0.135 | 0 | 0.0076 | 0.077 | 4.24 | 72 |
| 4 | 48 | 8.4 | 0.130 | 0 | 0.0062 | 0.110 | 1.60 | 72 |

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# EFFECT OF ENDO-PG TREATMENT ON THE MINERAL CONTENT OF RED BEET JUICE

K. ZETELAKI-HORVÁTH,[a], B. LAKATOS,[b] and NGUYEN XUAN THIEN[a]

[a]*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
[b]*Central Chemical Research Institute of the Hungarian Academy of Sciences, H-1525 Budapest, Pusztaszeri út 59–67. Hungary*

There are a lot of data in the literature on the beneficial effect of red beet in the preservation of health and in increasing the resistance of the human organism against various diseases. This is why red beets were used in our experiments as basic material of several vegetable and fruit cocktails.

Five red beet varieties (obtained by courtesy of the Vegetable Research Institute, Kecskemét) i.e. Detroit, Little Ball, Bíborhenger, Rote Kugel and Bordo OMFI were investigated. The concentration of Ca, Mg, K and Fe were determined in the beet tissues as well as in the juices obtained after enzymatic (endo-polygalacturonase, ZETELAKI-HORVÁTH et al., 1981) disintegration (ZETELAKI-HORVÁTH & VAS, 1980).

The highest Mg- and K-contents were found in the tissues of Rote Kugel (0.23 %) and Little Ball (3.7 %), while with respect to Fe-content variety Bordo OMFI proved to be the best.

The various red beet varieties were solubilized with 0.5 % (v/w) endo-polygalacturonase (Phylendonase, Phylaxia Budapest). The tissue–water ratio was 1:1, the pH: 3.8, and incubation was carried out for 1.5 h at 40 °C. After solubilization and enzyme inactivation (at 85 °C, 15 min) the pulps were strained, then the degree of disintegration and the recovery of minerals were determined.

The most effective enzymatic solubilization was obtained in the case of the varieties Bíborhenger and Detroit. The highest Mg-concentration (129 $\mu g\ cm^{-3}$) was measured in the juice of Rote Kugel. The Mg:Ca ratio was the highest in the juice of the varieties Rote Kugel and Detroit (1.55 and 1.4 %, resp.) while the lowest was found in Bordo OMFI. The highest K- and Fe-concentrations were determined in the juice of Bordo OMFI.

Three varieties (Bíborhenger, Rote Kugel and Bordo OMFI) were put at our disposal by the National Institute for Agricultural Variety Testing, Budapest) in a larger quantity for further investigations. The beet tissues showed a difference in composition. The highest protein content (16.3 $\pm$ 0.25 %) was measured in variety Bíborhenger, the highest carbohydrate-content (70 $\pm$ $\pm$ 3.2 %) in Rote Kugel.

The highest juice yield was obtained after the enzymatic solubilization of Bordo OMFI (106 % of the enzymatic non-treated reference sample), while the highest protein and carbohydrate yields were measured in Bíborhenger and Rote Kugel (117 $\pm$ 2.4 % of the reference samples).

Fibrous juices were prepared by enzyme treatment of the red beet variety Bordo OMFI and sour cherries (var. Pándi). The red beet and sour cherry juices were mixed in a ratio of 4:1 and were stored in 500 $cm^3$ bottles in the natural state (reference) and with the addition of Mg-oligogalacturonate (20 mg per 100 $cm^3$) at 15 °C for 1 year. After 1 year sedimentation in the reference bottles was 33 $\pm$ 2.1 %, in the samples containing Mg-oligogalacturonate only 14 $\pm$ 1.3 %.

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# MICROBIOLOGICAL STABILITY OF CARROT JUICE AS A FUNCTION OF HEAT TREATMENT

E. SZILÁGYI-TÓTH,[a] O. REICHART[b] and K. ZETELAKI-HORVÁTH[a]

[a]*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
[b]*Microbiology Department Group, University of Horticulture, H-1118 Budapest, Villányi út 35–38. Hungary*

At the Bioengineering Department of the Central Food Research Institute an enzymatic method was developed (utilizing endo-polygalacturonase) for the liquefaction of vegetables of high dry matter content (ZETELAKI-HORVÁTH & GÁTAI, 1977). The enzyme is manufactured by Phylaxia (Hungary) by the method developed at the Institute and marketed under the name Phylendonase.

The aim of the present study was to establish the parameters of mild heat treatment for nectars prepared by the enzymatic technology.

Carrots, one of the microbiologically most contaminated vegetables and apples were used in the experiments. Both carrots and apples were treated with Phylendonase (0.5 and 0.05%) at 80 °C. The carrot juice in itself and mixed with 50% apple juice were used as model substances.

The thermal death parameters were established for a mixed microflora by a new dynamic method (REICHART, 1979). The initial mesophilic aerobic viable cell count and its change during storage were characterized by the survival curve. The most important parameters of thermal death belonging to temperature T, the thermal death rate constants ($k$) were determined on the basis of the survival curve, while the decimal times ($D$) were established from the slope of the actual tangent of the curve.

The equations of the curves and the negative reciprocals ($z$) of the slope were calculated by linear regression from the related pairs of $\log D - T$ values.

For the sake of completeness the pH-dependence of $D$ and $z$ values were studied in the pH range of 3 to 7. Decimal times proved to be pH-dependent in the range of pH 3.0 – 5.5.

Pasteurization, at 80 °C for 0.5 min, of juices with pH values below 4.5 was found to be sufficient as regards their microbiological state. From the point of view of protecting the nutrients, vitamins and colour as well as the sensory quality the above treatment was highly significantly more advantageous than pasteurization for 25 min.

However, it has to be taken into account that the above heat treatment provides protection only against the vegetative forms of bacteria, yeasts and fungi. To protect the juices against spore forming bacteria the pH value has to be kept under the critical level and the temperature of heat treatment has to be above 100 °C.

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# CLARIFICATION AND PURIFICATION OF DATE EXTRACT WITH THE ENZYME PECTIN LYASE

K. KOVÁCS and M. NAGY-GASZTONYI

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

In North Africa and South-West Asia the date palm is a wide-spread plant. In Libya alone there are 90 million date palms yielding a produce of 1.25 million tons of dates per year. Dates contain more than 50% sugar and other carbohydrates, thus they are suitable to form the raw material of sugar manufacture. Date sugar is frequently processed in the form of syrup and is used as such in nutrition.

Date syrup of 67–72% solids content consists to 95% of reducing sugars. An important step in the chemical process of date syrup manufacture is the production of a clarified, transparent juice. At present date extract is clarified by precipitation, ion exchange and ultra-filtration processes.

In this study clarification and purification of the date extract was carried out by an enzymic method. A pectolytic enzyme of 3.17 $\mu$mol min$^{-1}$ cm$^{-3}$ pectin lyase activity was gained by fermentation from an *Asp. foetidus* species.

Enzyme action was studied in the aeqeous extract of comminuted dates by the change in transmission and reduction of viscosity as a function of enzyme concentration, treatment time and temperature as well as pH.

The transmission at 800 nm of the date extract increased from the initial 10% with increasing enzyme concentration to 75% at 30 °C and to 83–85% at 40–50 °C. The specific viscosity of the juice decreased from 5.1 to 2.8 at 50 °C at an enzyme concentration of 0.01 $\mu$mol min$^{-1}$ cm$^{-3}$.

A temperature of 45—50 °C was found ideal for the enzymic treatment of date extract. With 40 min treatment at 50 °C and 0.01 $\mu$mol min$^{-1}$ cm$^{-3}$ enzyme concentration transmission increased by 55—59%, while specific viscosity

decreased at 45 °C from 32.85 to 3.0 and at 50 °C from 20.55 to 3.5. The greatest increase in transmission and the greatest reduction in viscosity was achieved in the pH range of 4–5 as compared to the sample not treated with enzyme.

As proven by the experiments the pectin lyase preparation obtained by *Asp. foetidus* fermentation is suitable for the purification and clarification of date juice and by the application of this enzyme a transparent date juice can be obtained.

## STARCH HYDROLYSIS AND SUGAR PRODUCTS BY ENZYMATIC PROCESSES

L. Ludvig

*Research Institute of Alcohol Industry, H-1089 Budapest, Diószeghy Sámuel u. 8. Hungary*

The starch industry utilizes a great amount of enzyme preparations in the hydrolysis of starch and in the isomerization of glucose thus obtained.

Fructose corn syrup of high fructose content is produced from starch by the application in succession of different enzymatic procedures. The starch is solubilized and liquefied to about 15–20 dextrose equivalent (DE) with α-amylase of bacterial origin. Subsequently the solubilized starch is hydrolysed to glucose with glucoamylase. In the third step a part of the glucose is isomerized to fructose with glucose isomerase.

To optimalize the manufacturing technology it is necessary to have a thorough knowledge of the reaction kinetics of the individual enzyme preparations. Thus, the effects of temperature, pH, enzyme–substrate ratio, reaction time, solids content, activating and inhibiting substances have to be studied in batch and continuous systems. At the same time the effect of the quality of the raw material has to be continuously checked.

The glucose obtained by hydrolysis is of pharmaceutical quality and is suitable for the production of vitamin C. The glucose syrup of high fructose content (42–44 %) can replace beet sugar in many products of the food industry. The fructose content of the syrup can be raised by further technological processes to above 55 %. The glucose syrup of high fructose content is suitable for further processing by various techniques (ion-exchange, fractionated crystallization, etc.). Crystalline fructose can be made of it the sweetening capacity of which is about 15–20 % higher than that of saccharose.

# INCREASING THE UTILIZABILITY OF ACIDIC WHEY BY AN IMMOBILIZED LACTASE PREPARATION

V. NÁDUDVARI-MÁRKUS and E. KISS

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Three quarters of the quantity of cheese and curd whey produced in Hungary are utilized as such, further 10% in a processed form. The rest (15%) are lost and pollute the environment. In model experiments it has been established that the lactose content of curd whey formed from milk of 46 g $dm^{-3}$ average lactose content is 40 g $dm^{-3}$ (NICKERSON et al., 1976). The whey formed in the course of the production of the enzymatically pretreated milk protein hydrolyzate "Sportrobi" contains nearly all the lactose present in milk. One of the ways to utilize the lactose that otherwise would get lost, is lactose hydrolysis. This requires a degree of hydrolysis of about 70%.

In the experiments presented, acidic whey (pH 4.1–4.5) was treated with the preparation Lactase LP® (Rapidase, France) derived from *Asp. niger* and immobilized by adsorption and cross-linking with glutaraldehyde to the phenol-formaldehyde resin Duolite (Dia Prosim, France) (HYRKÄS et al., 1976). A surface was formed of the immobilized lactase by using a textile cloth. Thus the immobilized enzyme can be separated from the whey containing protein aggregates.

With the immobilized lactase preparation (12 g dry resin; 1070 U $g^{-1}$) 320 $cm^3$ acidic whey were hydrolyzed for 2 h, in the temperature range of 40–60 °C. The duration of hydrolysis was selected taking into account the industrial conditions and the hazard of microbial contamination. Lactose conversion increased with increasing temperature (from 28 to 56%). At 60 °C about 8 h are required, under the given conditions, for total conversion.

The hydrolysis of a 50 g $dm^{-3}$ lactose solution was performed in a column reactor filled with immobilized lactase (volume of the resin bed: 54 $cm^3$; 16 g dry resin; 1320 U $g^{-1}$), applying different substrate flow rates in the temperature range of 40–60 °C. In the column reactor the lactose solution could be entirely hydrolyzed at 60 °C and flow rates of 0.5–1.0 $cm^3$ $min^{-1}$.

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## COMPARISON OF DIGESTION RATES OF FOOD PROTEINS

G. Szabolcsi, N. D. Hung, E. Cseke and M. Vas

*Institute of Enzymology, Biological Research Center, Hungarian Academy of Sciences, H-1113 Budapest, Karolina út 29. Hungary*

The kinetics of tryptic digestion of food proteins (commercial soy meal, as well as casein and commercial milk powders) were studied by measuring

— the trichloroacetic acid soluble peptide release and

— peptide bond splitting

either by titration of the liberated protons at constant pH or by recording the pH drop in non-buffered suspensions. The theoretical basis of the pH drop assay was elaborated. We showed that application of this method for comparative studies required complementary determinations of buffering capacities of the samples and the time course of the reference protein digestion.

It was shown that relative digestion rates of food proteins could be calculated from the ratio of times required to attain the same degree of digestion (if peptide release or peptide bond splitting were determined) and from the ratio of times required to attain the same pH value, if the pH drop assay was used. The studies revealed that commercial milk powder preparations differed in digestion rates by a factor of two and various lots of commercial defatted soy meals differed as much as by a factor of three.

The effect of new processing technologies, devised to enhance the digestion rate of food proteins could be tested in vitro. Increase in the in vitro tryptic digestion rates was reflected in decreased feed consumption per weight gain in pilot plant pig feeding experiments.

## DETERMINATION BY COULOMETRIC TITRATION OF THE DEGREE OF PROTEOLYSIS

Á. Baráth and A. Halász

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Partially hydrolyzed proteins play an increasingly important role in nutrition biology and in utilization of by-products of the food industry as additives. The objective determination of the degree of proteolysis is an important requirement because thereby the control and design of the proteolytic process becomes possible.

The method used for the determination of the degree of proteolysis is the so called "controlled enzymatic hydrolysis". The essence of the method consists in carrying out the hydrolysis at the optimal pH of the enzyme applied

(usually neutral or slightly alkaline) and following up the process by the alkali consumption. The amount of alkali needed to keep the pH at a constant level is inversely proportional to the degree of hydrolysis. This can be defined as the proportion in % of split peptide bonds to the total number of peptide bonds.

The pH-stat system required for measurement can be provided by means of a continuous titrator. Measurements were carried out on an Acigraph OH-408 type apparatus (Radelkis, Budapest) which can be operated as a coulometric titrator. Thus, contrary to the customary titration, the titration reagent (NaOH) can be produced in the sample itself by electrolysis.

The inductive current serving to produce the reagent is introduced into the solution by way of the silver generator electrode pair. The reagent is formed at the negative pole of the electrode pair, while the acid formed at the positive pole which would interfer with titration, reacts with the silver ions and forms an insoluble silver-halogenide precipitate.

The amount of alkali produced by the inductive current is registered and can be read off. The degree of dissociation ($\alpha$) can be calculated from the amount of alkali used and the degree of hydrolysis from the total number of peptide bonds ($h_{tot}$) using the following equation:

$$DH = \frac{B \cdot N_B}{MP} \cdot \frac{1}{\alpha} \cdot \frac{1}{h_{tot}} \cdot 100\,\%$$

where

$DH$ = degree of hydrolysis
$B$ = amount of alkali used ($dm^3$)
$N_B$ = normality of the alkaline solution
$MP$ = mass of protein (kg)
$\alpha$ = degree of dissociation
$h_{tot}$ = total number of peptide bonds in the protein (gram equivalent per kg)

This coulometric titration method was used in model experiments of casein hydrolysis by pancreatin. The substrate and enzyme concentrations were taken into account as parameters of the enzyme reaction. The method proved to be well reproducible, the standard deviation of parallel measurements being 2–5%.

## INCORPORATION OF $^{14}C$ METHIONINE INTO THE PROTEOLYTIC HYDROLYSATE OF PROTEIN BY AN ENZYMATIC PROCEDURE

Gy. Hajós and T. Szarvas

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
*Isotope Institute of the Hungarian Academy of Sciences, H-1121 Budapest, Konkoly Thege M. út 29–33. Hungary*

The plastein reaction is a process of protein modification directed by enzyme catalysis which can be used for increasing the nutritive value of proteins by incorporation of essential amino acids. On the mechanism of this process and on the mode of incorporation of amino acids no common stand has been developed as yet in the literature.

In the course of these experiments methionine (S-methyl-$^{14}C$) was incorporated into the proteolytic hydrolysates of casein and serum-albumin by the plastein reaction. The incorporation of methionine was detected by determination of the radioactivity of the highest molecular mass fraction. Radioactivity of the precipitation obtained by addition of trichloro-acetic acid (10% TCA) showed that the radioactive methionine has been incorporated into the highest molecular mass fraction by covalent bonds.

This radioactive protein fraction of high molecular mass was fractionated by SDS-polyacrylamide gel electrophoresis. The radioactivity of the separated protein fractions was determined by a liquid scintillation spectrometer. Radioactivity was detected in a molecular mass range of 15–20 000 D and of 10 000 D in the case of casein, and at about 10 000 D with serum albumin.

The amino acids of the protein fraction precipitated by TCA were identified after hydrochloric acid hydrolysis by thin-layer chromatography. Radioactivity measurements were carried out over the whole of the layer and high activity was found at the spot corresponding to methionine (based on the $R_f$ value and control methionine).

Radioactivity was also measured at two additional spots, of two different $R_f$ values. Identification of these amino acids or amino acid derivatives is in progress.

## DETERMINATION OF TRYPSIN INHIBITOR ACTIVITY IN SOYA PRODUCTS

J. Petres

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

In Hungary one of the requirements in the utilization of soya products for human consumption is that their trypsin inhibitor activity (TI) should not exceed the specified level.

Therefore the investigations on soya utilization were extended over the adaptation of a method suitable for the determination of TI activity in Hungarian food control institutions while the results thus obtained would be comparable to those found in the related international literature.

The method developed by Kakade and co-workers appeared to be suitable for this purpose. It was applied in a form further improved by the Central Soya Company (USA) and kindly put at our disposal.

The principle of the method is that from the *N*-benzoyl-DL-arginine-*p*-nitroanilide (DL—BAPA) used as substrate, p-nitroanilin is liberated by trypsin under specified conditions. The light absorption of the reaction mixture, which is directly proportional to the quantity of the liberated *p*-nitroanilin, is measured at 410 nm. Because of the inactivation of part of the trypsin in the reaction mixture, due to the presence of the inhibitor, hydrolysis of the substrate is slowed down and the amount of liberated *p*-nitroanilin or, in other words, the absorbance of the mixture, is lower.

Kakade, on evaluating the results of measurements, converted the Trypsin Inhibitor Units (TIU) belonging to soya suspensions of different volumes to activity values belonging to 1 $cm^3$ suspensions. He found that with increasing inhibitor concentration the TIU values decreased. The values converted to 1 $cm^3$ (TIU per cm), when plotted against the initial volume of the suspension, were found to give a linear correlation. It was concluded from this observation that the actual trypsin inhibitor (TI) activity is best approximated if the curve connecting the TI activity units is extrapolated to 0 $cm^3$.

In this study instead of the graphical solution the more exact linear regression calculation was applied, where the TIU value of 1 $cm^3$ soya suspension is obtained from the linear regression equation ($y = a + bx$), in the case of $x = 0$ by the value $a$ (the constant in the equation).

If the correlation coefficient ($r$) is between 0.8 and 1 the TIU value belonging to 1 mg of the sample investigated is calculated from the value $a$. If value $r$ is lower than 0.8 then the mean of TIU values converted to 1 $cm^3$ is taken into account. The latter case occurs with highly heat treated samples of low inhibitor activity.

From the great number of experimental results it was concluded that the method can be used to determine the TI activity in addition to soya beans and soya products, in products prepared with soya. Carrying out the measurements in three parallels the TI activity can be established with a relative error not exceeding 4–10%.

# STUDY OF THE CORRELATION BETWEEN THE THERMOSTABLE DESOXYRIBONUCLEASE (TNASE) AND TOXIN PRODUCTION IN *STAPHYLOCOCCUS AUREUS*

GY. LOMBAI

*Institute of Food Hygiene, University of Veterinary Sciences, H-1400 Budapest, 7 P.O.Box 2. Hungary*

About 95% of staphylococci is capable of producing the extremely resistant enzyme desoxyribonuclease. The enzyme appears in every medium where *Staphylococcus aureus* grows. Enzyme production, similarly to toxin production, is affected by micro-ecological factors (temperature, pH, water activity).

Sufficiently sensitive methods are available for the detection of TNase, thus, used directly in foods, they show whether the food had been contaminated with *Staphylococcus aureus* and therefore may be hazardous. This method serves as a screening test and in negative cases it is reassuring to know that the danger of staphylococcus poisoning does not exist. On the other hand, because of the qualitative character of the test, it does not answer the question whether the growth of the strain or strains was accompanied by toxin production.

The aim of this study was to try and find out whether the quantity or activity of TNase give any information on the toxin production of the strain. The correlation was studied on 24 paired *Staphylococcus aureus* strains. Each pair was similar in their morphological, biochemical and phage-type characteristics and differed only in their toxin producing capacity. The strains were grown under oxygen injecton in a brain-heart broth. The TNase activity of the heat treated medium, cleaned by centrifuging, was measured against a substrate containing a known amount of DNA. The activity values were characterized by the quantity of DNA not decomposed as measured with a photometer.

The data were used in contingency calculations resulting in

$$\chi^2_{\text{calculated}} = 0.6713 < \chi^2_{\text{table}\begin{cases} f=1 \\ p=0.1 \end{cases}} = 2.706$$

This shows that the difference in the enzyme activity of toxin negative and positive strains is not significant.

To check this finding the two-sample $t$ test was also carried out:

$$t_{\text{calculated}} = 0.7444 < t_{\text{table}\begin{cases} f=1 \\ p=0.1 \end{cases}} = 1.717$$

Here, too, the difference between the two groups was non-significant.

Thus it was concluded that from *Staphylococcus aureus* strains capable of TNase production the toxinogen strains cannot be selected on the basis of enzyme activity measurement.

A quantitative limit permitting of drawing conclusions as to the wholesomeness of the given food cannot be laid down either. The TNase test serves as an accurate and useful qualitative screening test. In the case of a positive result, however, further investigation is needed. Since serological toxin detection is cumbersome, research has to be extended in the direction of biological tests.

## ENZYME-ENGINEERING IN STARCH PROCESSING

E. László

*Department of Agricultural Chemical Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

*Enzyme isolation — affinity chromatography*

The procedures presented so far are suitable for binding only 2–3 mg of enzymes per $cm^3$ of carrier-bed. This capacity must be raised 10–20 times for economical industrial scale enzyme production. The capacity can be increased only by determination of the thermodynamical parameters of enzyme-substrate binding. In the case of starch-hydrolyzing enzymes two methods are currently applied: determination of the individual rate constants and investigations into inhibition kinetics of the substrate-analogue.

*Production of modified enzymes for planned industrial starch-hydrolyzing technologies*

After clarifying the role of amino acid side chains, we determined the position of functional groups in the active centre by means of active centre mapping based on product distribution. In our assumption, modification at any site of the active centre will cause changes both in the product spectrum and in the active centre map. As an example, we present the results of the modification of tryptophil groups in *B. subtilis* alpha-amylase. The product spectrum changes upon tryptophil modification. The glucose and maltose ratios in the products increase. We named this promising technique: "enzyme surgery".

*Production of immobilized enzymes*

Diazo-coupling of the tyrosyl group produced 18% residual activity, while immobilization by means of isothiocyanate, which reacts with the free amino

group of the enzyme, gave an entirely inactive product. Considering the structure of the active centre of gluco-amylase, the failure of immobilization is due to the same reason as given for alpha-amylase.

*Application techniques of immobilized enzymes*

In our model experiments we applied *Asp. niger* gluco-amylase immobilized by *p*-benzoquinone to DEAE-cellulose (bead-form), and studied the degradation kinetics of industrial substrates in a continuous mixed tank reactor and a packed-bed reactor.

In the case of industrially applied immobilized glucose-isomerase it has been established that external diffusion hindrance is practically negligible and the decrease in the rate of the enzymatic reaction is primarily due to internal porous diffusion inhibition.

By presenting these results, we wished to show that enzyme engineering is to be regarded today as an integral part of biotechnology, which greatly promotes economical design and management.

## FUNCTIONAL GROUPS OF GLUCOSEISOMERASE AND MECHANISM OF ENZYMATIC ISOMERIZATION

E. László, Á. Hoschke, K. Balogh and V. Kajtár

*Department of Agricultural Chemical Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

Glucoseisomerase is the immobilized industrial enzyme preparation used in the greatest amount. Combined with other enzymes it can be used to produce 1:1 glucose–fructose syrup from starch as a starting material. The detection of basic relations in enzyme technology and the demand for more valuable preparations of higher fructose content necessitate a detailed knowledge of the structure and functional mechanism of the applied enzyme.

The functional groups of glucoseisomerase originating from *Streptomyces albus* were studied by chemical modification and the effect of modification was tested by a kinetic method. These histidyl groups were modified with diethyl pyrocarbonate and 6 out of 10 histidyl groups were found to be modified, the rest being of "latent" character. The reactivity of the 6 histidyl groups was approximately identical and on the average the modification of one histidyl group lead to a 80% decrease in activity.

The amino groups were modified with trinitrobenzenesulfonic acid and 5 out of the 10 amino groups entered into reaction. The reactivity of the amino groups was identical and on the average the modification of 3 amino groups reduced the activity by about 30–40%.

The tryptophyl (modified with 2-hydroxy-5-nitrobenzylbromide), the tyrosyl (modified with *N*-acetylimidazol) and arginyl (modified with 2,3-butanedione trimer) groups of the enzyme are not functional in the enzymatic catalysis.

The mechanism of isomerization was studied by the binding affinity of cyclic substrates (glucose-fructose, xylose-xylulose, 5-deoxy-xylose), by substrate analogues (1-deoxy-1-amino fructose) as well as acyclic substrates and substrate analogues (e.g. 5-deoxy-xylulose).

## SEPARATION OF THE FRACTIONS OF DIFFERENT ACTIVITIES OF A CELLULOLYTIC ENZYME PREPARATION

M. Szakács-Dobozi, Gy. Hajós and L. Vámos-Vigyázó

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Within the frame of a long term cooperation agreement between the Institute of Technical Chemistry of the Academy of Sciences of the German Democratic Republic, Leipzig, and the Central Food Research Institute, Budapest, the authors carried out experiments to separate the components of the cellulase preparation of Gliocladium origin produced at the former institute by fermentation.

The isoelectric points (pI) of the components of the enzyme preparation were determined, after desalting on a Sephadex G-25 column, by analytical flat-bed isoelectric focusing in a Pharmacia (Uppsala, Sweden) apparatus. As the pI-s of the 31 components separated were in the pH-range of 9.05–2.4, a 3–10 pH gradient was established with Ampholine for preparative separation of the enzyme components. At the end of the run the gel was cut into 28 bands and, after pH-measurement, the protein content was eluted with distilled water. The activities of the fractions were determined by various methods: filter paper degrading activity (FPA); cotton degrading, i.e. $C_1$ activity, carboxymethyl cellulose degrading, the so-called $C_x$ activity as well as cellobiose degrading activity (Mandels et al., 1976).

The $C_x$ and cellobiase activities were nearly recovered in the fractions, in some of them in a pure state, while the recovery of FPA was only partial and $C_1$ activity was found in traces only. Recombination experiments were performed to recover $C_1$ activity: combining pure $C_x$ and cellobiase components (1:1) yielded in some instance considerable increases in $C_1$ activity. The most marked increase (19-fold) was observed by mixing the $C_x$ component of pI 4.39 and the cellobiase component of pI 8.19.

In order to determine the molecular masses of the individual enzyme components, flat-bed SDS-polyacrylamide gel electrophoresis was applied (SDS =

sodium dodecyl sulfate) (WEBER & OSBORN, 1969). The protein bands separated were detected by silver staining. The molecular masses of the enzyme components fell into the range of 11 000 to 15 000. Several components of different pI-s but identical activity had also identical molecular masses. The enzyme components in the pH-range of 4.19 to 9.75 stained yellow with $AgNO_3$. This suggests the presence of glycoproteins.

The results presented support the assumption reported according to which the activities FPA and $C_1$ play an initiative role in the mechanism of cellulase action and the synergistic effect of all the components is needed for successful cellulose hydrolysis (WOOD and MCCRAE, 1977). On the other hand, the enzyme components in the pH-range of 4.19–9.75 show $C_x$ activity and this activity has been reported to be linked, in general, to glycoproteins (BERGHEM, 1975).

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# EFFECT OF pH AND TEMPERATURE ON THE ACTIVITY OF VARIOUS PECTOLYTIC ENZYMES

M. NAGY-GASZTONYI and K. ZETELAKI-HORVÁTH

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Pectolytic enzymes are largely used in the food industries. Depending on the aim of technology clarifying or macerating enzyme preparations are used.

The aim of the present study was to compare the pH and temperature optima of enzyme preparations of various origin.

A clarifying enzyme from *Asp. foetidus* (PG-227), a macerating type enzyme from *Asp. awamori* (PG-225), both prepared at the Bioengineering Department of the Central Food Research Institute, further two preparations of Phylaxia (Budapest) manufactured on the basis of the technique developed at this Institute, one of them of clarifying, the other of macerating type (Phylazime and Phylendonase, respectively) were compared with Pectinol fest (Röhm, GFR) and Ultrazym (Ciba-Geigy, Switzerland) preparations of clarifying type and Rohament-P (Röhm, GFR) of macerating type, all three of foreign origin.

The endo-PG, endo-PMG and apple juice clarifying activities of the preparations were established by the measurement of their viscosity reducing capacity. The optimum pH of all the preparations was found to be in the pH range of 4–5. The high endo-PG activity, characteristic of macerating type enzymes, was observed with both preparations, PG-225 and Rohament-P, at pH = 4.5 ($2\times10^6$–$4\times10^6$ $dm^3\ h^{-1}\ dm^{-3}$). The endo-PG activity peak of the clarifying enzymes (PG-227, Pectinol-fest, Ultrazym) was again at pH = 4.5, however, substantially lower ($10\times10^3$–$800\times10^5$ $dm^3\ h^{-1}\ dm^{-3}$). The apple clarifying activity of PG-227 reached the highest value at pH = 5 (40 $\mu$mol $min^{-1}\ g^{-1}$) while the endo-PMG activity at pH = 4 ($4.3\times10^8$ $dm^3\ h^{-1}\ l^{-1}$).

The maximum of endo-PG activity of all the preparations was found at 50 °C and that of endo-PMG activity at 40 °C.

## INACTIVATION OF VEGETABLE PEROXIDASE UNDER MILD CONDITIONS OF DEHYDRATION

L. Vámos-Vigyázó

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

Within the vast amount of communications on peroxidase (POD) inactivation no data are available on the changes in activity of the enzyme in vegetables during dehydration. The increasing interest in dehydrated foods makes investigations in this direction indicated.

Preliminary experiments carried out with a number of commercial dehydrated vegetables showed a wide variation in POD activity (Table 1).

Table 1

*Peroxidase activity in commercial dehydrated vegetables*

| Name of dehydrated product | pH | Moisture content (% w/w) | Activity related to solids content (kU $g^{-1}$) | |
|---|---|---|---|---|
| | | | $\bar{x}$ | $\pm s$ |
| Potato (flakes) | 5.9 | 5.9 | — | — |
| Onion (sliced) | 5.8 | 2.5 | 6.20 | 0.243 |
| Parsnip (diced) | 6.2 | 6.0 | 25.1 | 0.879 |
| Celery (diced) | 5.5 | 6.1 | 56.7 | 2.27 |
| Horseradish (flakes) | 5.0 | 6.8 | 76.0 | 3.75 |

1 kU = 1000 U; 1 U = $10^{-3}$ $\Delta A_{420}$ $min^{-1}$ (Mihályi & Vámos-Vigyázó, 1975)
— = not measurable
$\bar{x}$ = mean value
$\pm s$ = standard deviation

The kinetics of moisture loss and POD inactivation during drying were studied in laboratory model experiments using diced celery (1 cm edges). This vegetable was selected because no regeneration of its POD activity had been observed within 2 h following 10-min heat treatment of its homogenates at 65 °C, 85 °C and 90 °C. The respective losses of activity as related to the value found in the untreated homogenate were 1.2 %, 87 %, 97 % and 99 %, thus 65 °C was selected as a relatively mild drying temperature at which inactivation might be assumed to be primarily due to the decrease in moisture content.

During the 240-min drying period moisture content was reduced to 16 % and POD activity to 13 % of the initial value. Moisture loss proved to be a first order kinetic process.

POD inactivation occurred according to biphasic first order kinetics, whereby the first phase took 40 min. In the two phases losses of moisture amounted to 32 % and 52 % and those of activity to 72 % and 15 %, respectively, as related to the initial value.

It was concluded that under mild conditions of dehydration the inactivation of POD in vegetable tissues occurred in a way similar to that observed at constant moisture content (SCHMIDT & VÁMOS-VIGYÁZÓ, 1981).

## Literature

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# UTILIZATION OF ENZYMATIC METHODS IN FOOD ANALYSIS

D. TÖRLEY

*Department of Biochemistry and Food Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

The sensitivity of enzymatic analysis is very high. With photometric methods micromoles of substances may be determined in a sample, whereas the order of magnitude of the enzymatic analysis means nanomoles. This sensitivity may be increased, by the utilization of fluorometry, by another 2–3 orders of magnitude.

As for the accuracy of enzymatic methods in the case of simple determinations of metabolites the coefficient of variation is less than ± 1 %, in the determination of enzyme activities generally ± 2–4 %, in coupled reactions ± 6 %.

As a disadvantage of the enzymatic analysis its high expenses are often mentioned. Though it is indisputable that the costs of the reagents are generally 3–5 times higher than with traditional methods, but if wages of working hours are calculated, too, then the enzymatic analysis is less expensive as the working time needed is only the 0,1–0,2th of that of the classical methods.

Enzymatic analysis may be used in the following fields of food analysis:

— the inactivation of enzymes at higher temperatures has been used for a long time past to control the proper heating applied during pasteurization or sterilization. By determining the residual activity of one or more enzymes we can establish whether the denaturation of the enzymes took place at the required level or not;

— from the measurement of the activity of the exoenzymes produced by microorganisms it is possible to draw conclusions as to the microbial contamination or spoilage of some foods;

— at present the number of enzymatic methods used for the elucidation of the composition of foods is considerably increasing. This phenomenon cannot be attributed only to the increased availability of commercial enzyme preparations but also to the enormous development of the techniques of enzymatic analysis i.e. automation, the use of immobilized enzymes, coenzymes, enzyme electrodes, etc. For the determination of some food components as many as 10–15 enzymatic methods are available. If the determination of the carbohydrates is taken as an example, the sugars are determined in most cases in the form of glucose. For this purpose two basic reactions are available:

$$\beta\text{-D-glucose} + O_2 \xrightarrow{\text{glucose oxidase}} \text{D-glucono-}\delta\text{-lactone} + H_2O_2 \qquad (1)$$

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{ADP} + \text{glucose-6-phosphate(G-6-P)} \qquad (2)$$

For example:

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{G-6-P dehydrogenase}} \text{D-glucono-}\delta\text{-lactone-P} + \text{NADPH} + H^+$$

Hydrogen peroxide formed in reaction (1) may be determined photometrically by the utilization of chromogenous substances, e.g. *o*-dianisidine, *o*-tolidine, 2,2'-diazobis-(3-ethylbenzothiazoline-6-sulphonic acid), etc. More recent methods are amperometric, potentiometric, polarographic ones, or dissolved or immobilized glucose oxidase, flow-through oxygen detectors, oxygen electrodes, autoanalyzers, etc., are utilized.

Reaction (1) is also suitable for the determination of the quantity of anomers: $\beta$-D-glucose may be determined with an oxygen electrode, then by adding mutarotase $\alpha$-glucose is transformed and determined as the $\beta$-anomer.

When using reaction (2) the sugar content may be determined by measuring the quantity of the NADPH formed, either at 340 nm by spectrophotometry or at 485 nm by fluorometry. In reaction (2) hexokinase may be substituted by

acylphosphate: D-glucose-6-phosphotransferase. In this case benzoylphosphate, nicotyl phosphate, acetyl phosphate, carbamoyl phosphate etc. may be used as phosphate donors.

By the aforementioned reactions the greatest part of the carbohydrates (mono-, oligo- and polysaccharides) may be determined in the form of glucose by using carbohydrases, isomerases. For example in a mixture of glucose, fructose, maltose, sucrose and starch we determine in the first sample the free glucose content by reaction (2), and afterwards by adding glucose phosphate isomerase the fructose content, too. In the second sample sucrose is hydrolyzed by invertase and the sum of free glucose and of glucose liberated from sucrose is determined by reaction (2). In the third sample maltose and sucrose are split by α-glucosidase and the glucose released is determined together with the free glucose. From these data the quantity of the monosaccharides and disaccharides may be calculated. In the next sample starch is hydrolyzed enzymatically and determined as glucose. Only the determination of lactose is more simple by utilizing β-galactose oxydase after hydrolysis with β-galactosidase. The accuracy of these determinations is fair, for components amounting only to 20% of the total carbohydrates the coefficient of variation is less than ± 2%. If the concentration of the monosaccharides is the tenfold of the disaccharides, the coefficient of variation for the latter is less than ± 4%. The accuracy decreases only when extremely high (100×) glucose concentrations are present.

— The number of enzyme preparations added to foods during different technological steps is permanently increasing and it is the task of the analysts to establish the activity and the usefulness of the individual preparations.

— The number of the known naturally occurring enzyme inhibitors is also increasing, the presence and inactivation of which is also determined by enzymatic analysis.

— Recent trend in food chemistry is the investigation of the tissue enzymes of foods. By studying the activity of some enzymes and isoenzymes it is possible, on the one hand, to get an insight into the biochemistry and biodynamics of foods, on the other we may get information on the usefulness, condition, state, etc. of the food in question.

From the above-mentioned examples it can be seen that in enzymatic analysis there are manyfold possibilities and variations even in such a narrow field like carbohydrate determinations. But there are also a great number of elaborated and widely used methods in food analysis for the determination of various other substances.

# APPLICATION OF SPECTROPHOTOMETRIC AUTOMATIC ANALYSIS SYSTEM IN FOOD ANALYSIS

Á. Hoschke, M. Polacsek-Rácz and J. Hank-Nyulász

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The automatic analyzer system made by Pye Unicam (U.K.) consists of an AC1 type programmable chemical unit, an SP6-550 UV/VIS spectrophotometer, a PM 8251 type one-channel recorder and an HP 97/S calculator. The enzyme analyzer can be applied with great possibilities of variation for end-point analytical measurements (15–240 samples per h, 20–70 °C and 0.15–1.50 $cm^3$ reaction volume), both in research and routine work.

The applicability of the analyzer will be presented on an example of fundamental importance in food analysis, i.e. enzymatic glucose determination. In developing the continuous method, the manual method of Afanasieva and Shcherbukhin (1975), a photometric procedure applying the enzyme system glucose oxidase — peroxidase and potassium cyanoferrate as hydrogen donor, was adapted.

The method developed permits of performing the analyses of 120 samples per hour at 37 °C and 20 min reaction period. Sample volume was 0.03 $cm^3$, the volume of the reagent 1.5 $cm^3$. Measurements were carried out at 410 nm. In the concentration range of 0.2–2.0 mg glucose $cm^{-3}$ the relationship between substrate concentration and absorbance was linear. The accuracy of the method, the variation coefficient was $\pm 0.5\%$. The results of the measurements have proven that the analyzer lends itself to accurate determination of the glucose content of a great number of samples. A further advantage of the method consists in the fact that — owing to the operation principle of the analyzer — the amounts of reagents and enzymes used can be greatly reduced. By its application, the cost of analysis can be reduced by nearly 50% as compared to glucose determinations in batch and continuous flow systems.

By combining glucose determination with other specific carbohydrate decomposing enzymatic pretreatments (glucoamylase, $\beta$-galactosidase, $\alpha$-glucosidase, invertase), the method developed was successfully applied to determinations of starch, maltodextrin, lactose, maltose and sucrose contents of food raw materials.

## Literature

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## COMBINED ENZYME ELECTRODES

J. Halbauer, B. Novák and E. László

*Department for Agricultural Chemical Technology, Technical University, Budapest, H-1521 Budapest. Hungary*

Analyses based on the specificity of enzymes play nowadays an important role in the qualification of foods, in monitoring food industrial processes and in food characterization. The development and use of enzyme electrodes in process monitoring is particularly important.

A glucose-maltose electrode was developed, suitable for utilization in continuous monitoring of the brewing process. The glucose electrode is made by Radelkisz (Budapest) with a glucose oxidase membrane. The maltose electrode contains beside glucose oxidase non-rate-limiting amounts of α-glucosidase.

The equation of the linear calibration curves of the electrode are as follows:

$$\text{for maltose: } I\ (\text{n A}) = 0.61 + 0.12\, C_M(\mu\text{mol dm}^{-3}) \qquad r^2 = 0.9982$$

$$\text{for glucose: } I\ (\text{n A}) = -1.000 + 0.1600\, C_G(\mu\text{mol dm}^{-3}) \qquad r^2 = 0.9999$$

The reproducibility of maltose measurements is higher than 10%.

The range of determinations:

100–600 μmol glucose per $dm^3$
100–800 μmol maltose per $dm^3$

The time required to reach 90% of the sign maximum ($t_{90}$):

for glucose: $t_{90} = 2.9$ min
for maltose: $t_{90} = 5.1$ min

## INVESTIGATIONS INTO THE SUGAR COMPOSITION OF HUNGARIAN RED PEPPER (PAPRIKA) DURING MATURATION AND STORAGE

M. Polacsek-Rácz[a], M. P. Pauli[b], L. Vámos-Vigyázó[a], and Gy. Horváth[a]

[a]*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*
[b]*County Station for Veterinary Hygiene and Food Control, H-6001, Kecskemét, Széchenyi krt. 29. Hungary*

Red pepper is an important export article of Hungary. Its characteristic taste is influenced, beside essential oils and colouring matter, also by the sugar content and — in the case of hot (pungent) paprika — by capsaicin concentra-

tion. The greatest part of sugar content is contained in the skin of the paprika pods. The solids content of the fresh pods ranges from 26 % to 54 %, according to cultivar. In the comparative investigations presented only paprika skin was used. Sugar composition was determined by methods of enzymatic analysis (ANON, 1979; BERGMEYER et al., 1970; POLACSEK-RÁCZ et al., 1981). The paprika samples investigated belonged to determined cultivars and came from the two most important locations of cultivation i.e. the regions of Szeged and Kalocsa, respectively.

The characteristic sugar components of fresh ripe paprika skin are glucose and fructose, Their ratio is — independently from cultivar — fairly stable, 1:1.2–1.5 (on the basis of 13 samples belonging to 10 cultivars). Saccharose is, in general, not present in the skin of ripe paprika. Of the 13 samples investigated only one contained sucrose, amounting to 2.7 % of the total sugar content.

Contrarily, in unripe, still green paprika skin the dominating sugar component was glucose. It amounted to about 60 % of the total sugar content, and sucrose was also found in considerable concentrations (16 % of the total sugar content). During ripening and transition into the red state, the total sugar content increased to about treble the value found in green pods. Within the total sugar content especially the fructose concentration increased to a considerable extent (7–fold), while the sucrose content decreased.

During storage of ripe paprika, the sugar content gradually decreased. The decrease was particularly marked in glucose content, thus the glucose — fructose ratio shifted more and more towards fructose, and sucrose reappeared among the sugar components. Thus sugar composition characteristic of paprika dried in the traditional way (in strings at ambient temperature) was gradually formed. Although the total sugar content as related to solids content varied with the cultivar (24–38 %), the ratio of the sugars was practically constant. Paprika dried in the traditional way always contained, beside glucose and fructose, also considerable concentrations of sucrose. On the basis of 7 samples belonging to different cultivars the percentage ratio of the three sugars glucose– fructose–sucrose was 26.5:56:17.5, or, related to glucose 1:2.1:0.67.

The sugar content of ground paprika skin obtained by industrial drying at elevated temperatures (in a Binder-type drier) amounted to about two thirds of that found in samples dried in the traditional way. Their sugar composition was also different. These paprika powders did not contain sucrose as the pods were dried shortly after picking and there was no time for sucrose synthesis. Glucose proved to be the sugar component more sensitive to heat treatment: its concentration decreased in the Binder-type drier more markedly in the phase of the drying procedure in which also humidity dropped suddenly.

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# DETERMINATION OF POLYPHENOL OXIDASE ACTIVITY IN PLUMS USING COUPLED REACTIONS

I. SCHUSTER-GAJZÁGÓ, Á. HOSCHKE and L. VÁMOS-VIGYÁZÓ

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The great variability of the enzyme polyphenol oxidase (PPO) in fruits makes it necessary to adapt the activity assay methods to the properties of at least the individual genera. Optimum conditions of measurement were established for quickfrozen plums applying the method developed by SUSSMANN (1961).

According to the underlying principle of the method an easily oxidizable compound, e.g. ascorbic acid or $K_4$ [$Fe(CN)_6$], is added to the reaction mixture which gets oxidized by the quinones, the primary products formed from the substrate upon the action of PPO, whereby the substrate is continuously restored from the quinones. The progress of the reaction can be followed by measuring the absorbance, at 420 nm, of the $K_3$ [$Fe(CN)_6$] formed.

Enzyme extracts were prepared as follows: 10 to 30 g of comminuted plum mesocarp were diluted to 100 $cm^3$ in *a)* 0.2 mol $dm^{-3}$ citrate buffer, pH 4.0 and *b)* the same buffer containing 10 g $dm^{-3}$ Triton $\times$ 100. Both suspensions were homogenized for 1 min with an Ultra Turrax homogenizer (Janke & Kunkel KG, IKA Werk Staufen Bresgau, FRG) then filtered.

The reaction mixture contained 2.8 $cm^3$ 0.04 mol $dm^{-3}$ $K_4$ [$Fe(CN)_6$)], 0.1 $cm^3$ substrate solution and 0.1 $cm^3$ enzyme extract. The blank contained the above citrate buffer instead of the enzyme extract. Substrate concentration in the reaction mixture varied from 0.09 mmol $dm^{-3}$ to 0.94 mmol $dm^{-3}$ chlorogenic acid and pH between 4.5 and 6.5. The enzyme reaction was performed at room temperature. Absorbance was continuously recorded on a spectrophotometer. The initial rate of changes in absorbance, i.e. enzyme activity, was computed by linear regression analysis. A change in absorbance of $1 \times 10^{-4}$ per min was adopted as unit enzyme activity.

The relationship between substrate concentration and reaction rate was hyperbolic, substrate saturation was reached at 0.94 mmol $dm^{-3}$ chlorogenic

acid concentration. The activity – pH curve had a plateau between pH 4.5 and 5, a local minimum between pH 5.5 and 6.0 and rose again till the end of the range of measurements (pH 6.5). The relationship between enzyme concentration and reaction rate was linear, if prepared according to *a)* in the range of the plum extract concentrations indicated above.

The conditions adopted for activity measurements were: 0.94 mmol $dm^{-3}$ chlorogenic acid in the reaction mixture, and pH 5.

When extracted with buffer containing Triton $\times 100$, activity values were about double as with citrate buffer only. This shows a considerable part of PPO in quickfrozen plums (picked at processing maturity) to be cell-bound.

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# DEVELOPMENT OF A METHOD FOR THE ASSAY OF LIPOXYGENASE IN DURUM WHEATS

K. CZULEK, A. PÁRKÁNY-GYÁRFÁS and L. VÁMOS-VIGYÁZÓ

*Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary*

The enzyme lipoxygenase present in durum wheats plays a role in decolourization of their yellow pigments (ESKIN et al., 1977; NICOLAS et al., 1982). Thus the values of lipoxygenase activity in durum wheats might be a tool in predicting the colour of pasta produced from them.

A method previously adapted from the literature for the assay of this enzyme in *T. aestivum* (PÁLOSI-SZÁNTHÓ et al., 1981) was further modified for the use with water extracts of *T. durum*. Linoleic acid was used as substrate and the changes in absorbance at 234 nm of the reaction mixture were followed during the enzyme reaction performed at 25 °C. These changes are proportional to the amount of compounds with conjugated double bonds formed as secondary reaction products.

Substrate concentration and pH were varied over the ranges of $2.67 \times 10^{-4}$–$2.67 \times 10^{-5}$ mol $dm^{-3}$ linoleic acid and pH 3.0–9.0, respectively, to establish optimum conditions for measurement. Reaction rate was found to vary with linoleic acid concentration according to a saturation hyperbola. Substrate saturation was reached at $1.33 \times 10^{-4}$ mol $dm^{-3}$ linoleic acid and this concentration was used in further measurements. The activity – pH curve had two local maxima, at pH 6.0 and pH 8.0, respectively. The former maximum was higher therefore pH 6.0 was adopted for the reaction mixture. In the range of

Table 1

Comparison of the parameters of lipoxygenase activity determination in *T. aestivum* and *T. durum*

| Measured parameters | Parameters of measurements | |
|---|---|---|
| | *T. aestivum* | *T. durum* |
| Wavelength (nm) | 234 | 234 |
| Temperature (°C) | 25 | 25 |
| pH | 6.9 | 6.0 |
| Substrate concentration (mol $dm^{-3}$) | $5.4 \times 10^{-5}$ | $1.3 \times 10^{-4}$ |

0.02 mol $dm^{-3}$ – 0.50 mol $dm^{-3}$, the ion concentration of the buffer did not affect the reaction rate, thus 0.1 molar buffers were applied throughout. The relationship between reaction rate and enzyme concentration was tested and found to be linear in the range corresponding to 2.7–6.7 g $dm^{-3}$ wheat. In the above conditions of measurement the changes in absorbance were found to be proportional to time up to 4 min.

Activity was calculated from the linear section of the absorbance (A) vs. time curve and related to 1 g of wheat; $10^{-3}\,\Delta A\ min^{-1}$ was adopted as unit activity.

The measured parameters, adopted for the kinetic spectrophotometric assays of lipoxygenase in the two kinds of wheat are compared in Table 1.

In assays of lipoxygenase in durum wheat samples of three varieties (GK Basa, GK Minaret, GK Madur) grown at three different locations (Tordas, Jászboldogháza, Iregszemcse) the variation coefficient of three replicates each was found to vary between 1.3 and 4.8%. Analysis of variance revealed significant differences (P = 5%) between the activities of wheats of different cultivars grown at the same location and of samples of the same cultivar grown at different locations. The highest and lowest activity values found in the nine samples were 20.2 ± 0.4 kU $g^{-1}$ (GK Basa, Tordas) and 0.22 ± 0.01 kU $g^{-1}$ (GK Madur, Iregszemcse) (kU = 1000 units).

## Literature

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# BOOK REVIEWS

C. SEBASTIO

## Igiene e controllo sanitario dei prodotti della pesca

## (Hygiene and sanitary control of fishing industry)

Editrice Impremare, Taranto, 1980, 616 pages

The book contains detailed and profound information on the products of sea and fresh water fishing. The anatomy, exact identification according to FAO recommendations of edible fishing products of plant and animal origin, are described. The processing of these products by the food industry and their sanitary control are also reviewed.

An account is given of the bacteriological, parazitological and toxicological health hazards involved by eating infected sea products or poisoned fish. Methods of bacteriology, chemistry and toxicology are described including the necessary media and reagents. The book contains the description of deseases of edible organisms as well as the changes occurring during the period elapsing between their fishing and marketing. According to their state of freshness the products fall into different groups.

All Italian laws and regulation pertinent to fishing, marketing of sea products, their processing, grading and sanitary control are given in the book.

The large amount of information contained in the book is valuable to the practising physician, bacteriologist, parasitologist, specialists of the food industry and even to the layman particularly interested in sea and fresh water animals.

W. KÖRBLER

TSVETAN TSVETKOV

## Spravochnik po kriobiologiya

## (Handbook of cryobiology)

Zamizdat, Sofia, 1983, 316 pages

The present handbook is dedicated to a new field of science — cryobiology — a field of enormous emotional charge and practical importance.

In the last decades cryobiology made fast progress due to recent results of cryophysics and cryogenics. The handbook considers the thermodynamical basis of cryogenics, properties of working substances and the basic cryogenic equipments applied in cryobiology practice.

Fundamental physico-chemical principles of water structure and the properties of water in various solutions, biosystems and food products are presented. Low temperature crystallization of water in biomaterials is discussed together with the fundamental types of cryoprotectors used for cryopreservation. Basic cryoinstruments and apparatuses applied in medicine, in particular, in surgery, are also accounted for.

The problem of freeze-drying of biomaterials and the equipments for its realization vacuum freeze driers — are fully elucidated. The fundamental concepts and terminology in cryobiology is listed.

The handbook will be of use to the broadest circle of specialists, working in the field of low temperature preservation of biomaterials.

R. LÁSZTITY

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# EFFECT OF FREE AMINO ACIDS OF THE GRAPE ON THE DEVELOPMENT OF ORGANOLEPTIC PROPERTIES OF WINE

OLGA JUHÁSZ[a] and D. TÖRLEY[b]

[a] Institute for Vinegrowing, University of Horticulture, H-1118 Budapest, Ménesi út 44. Hungary

[b] Department of Biochemistry and Food Technology, Technical University Budapest, H-1521 Budapest. Hungary

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Relationship between organoleptic quality and the free amino acids occurring in greatest amounts in the grapes of some varieties of *Vitis vinifera* L., was investigated.

The experiments were carried out on the one hand with must samples of normally fertilized Rizlingszilváni (Müller Thurgau), Ezerjó (Tausendgute), Hárslevelű (Feuille de tilleul) and Olaszrizling (Wälschriesling), and Rizlingszilváni fertilized with surplus nitrogen and potassium, on the other hand with wine samples of the Rizlingszilváni variety.

The amino acids were determined on an automatic amino acid analyser, the organoleptic evaluation of the wines was performed by a 20 score method.

In the must samples arginine and proline occurred most abundantly as free amino acids. A positive correlation was found between the accumulation of proline and the sugar–acid ratio at the P = 99% probability level. Authors presume that arginine influences adversely the development of the organoleptic quality of wines.

As to the effect of fertilization the development of the organoleptic properties of wines was positively affected by the use of surplus potassium, while on the contrary surplus nitrogen fertilizer exerted a negative effect.

**Keywords:** wine amino acids, grape amino acids, wine quality

The quality of the vintage of different grape varieties was hitherto characterized mainly by the sugar and acid content. In the determination of the maturity index the sugar–acid ratio is mostly used, too. FREGONI (1977) called already attention to the troubles related to the parameters used in the characterization of quality of grapes and wine and emphasized that the conventional parameters do not satisfy the actual quality requirements of musts and wines, nor does the influence of the soil.

With the development of methods of separation and analysis the investigations were recently extended over the study of other components of the grape, which could be used for the characterization of the technical degree of maturity, and above all the quality of the vintage. Of the organic compounds of grapes nitrogen containing compounds play an important role beside acids and sugars.

Research work hitherto done has shown that free amino acids are primordial in the metabolism of wine, in the determination of the biological value of grape and wine, and they are nutrients of the yeast and moulds, influencing the rate of fermentation. Amino acids are often primary materials of biosynthesis of plant metabolites and consequently they have a direct or indirect effect on the organoleptic quality of must and wine.

In previous papers (JUHÁSZ & POLYÁK, 1976; JUHÁSZ et al., 1977; MIKLÓS-JUHÁSZ & TÖRLEY, 1979) results of the investigations on the nitrogen containing compounds of grape were published. This paper deals with the correlation of the organoleptic properties of wine and of the qualitative and quantitative changes of free amino acids in must and wine.

## 1. Materials and methods

Experiments were carried out at Szigetcsép at the Experimental Station of the University of Horticulture on 200 $m^2$ parcels in 5 repetition with *Vitis vinifera* L., cultivar Rizlingszilváni syn. Müller Thurgau, Ezerjó syn. Tausendgute, Hárslevelű syn. Lipovina, Olasz rizling syn. Wälschriesling vine varieties, which were planted in 1966.

The basic amounts of fertilizers per hectare used before plantation were: 600 kg $K_2O$; 200 kg $P_2O_5$; 100 kg N, respectively.

Fertilizers per hectar yearly added were: 150 kg $K_2O$; 80 kg $P_2O_5$; 80 kg N, respectively.

Fertilizer per hectare distributed in surplus: 200 kg $K_2O$; 100 kg N, respectively.

Grape samples were taken every two weeks from the middle of July till the end of the maturation period. Sampling and other methods applied were described in previous papers (JUHÁSZ & POLYÁK, 1976; JUHÁSZ et al., 1977). Additional investigations included the analysis of ammonia, nitrate and organic nitrogen content (JUHÁSZ & GYULAI, 1980), the determination of the titratable acidity and the tartaric acid content, the sugar content by refractometry and the pH value (KÁDÁR, 1973).

Free amino acids were determined partly by thin-layer chromatography, partly by autoanalyser type AAA-881, made by Mikrotechna, Prague, Czechoslovakia (DÉVÉNYI et al., 1971).

Organoleptic examination was carried out by 16 wine panelists on Rizlingszilváni wines from the years 1977 and 1978 using a 20 score method (colour 0–2, limpidity 0–2, odour 0–4, flavour and general impression 0–12).

## 2. Results

Of the 23 amino acids which were identified by the amino acid autoanalyser in the must, arginine and proline were present in the greatest amounts (Tables 1–3). The arginine content increased 5–10-fold from the green berry

Table 1

*Composition of free amino acids in the musts of untreated and treated grape varieties harvested on 21st September 1976*
(mg per 100 $cm^3$)

| Free amino acids | Rizlingszilváni | | | Ezerjó | Hárslevelű | Olaszrizling |
|---|---|---|---|---|---|---|
| | Ø | N | K | | untreated | |
| Asp | 9.32 | 6.83 | 3.42 | 5.79 | 2.92 | 3.27 |
| Thr | 20.80 | 17.08 | 8.35 | 8.73 | 19.88 | 9.80 |
| Ser | 21.71 | 19.47 | 17.70 | 9.98 | | 15.50 |
| Glu | 18.36 | 16.60 | 15.66 | 15.54 | 10.92 | 14.44 |
| Pro | 16.38 | 18.00 | 17.13 | 14.50 | 8.73 | + |
| Gly | + | 0.86 | + | + | + | + |
| α-Ala | 16.90 | 17.80 | 6.61 | 14.80 | 9.80 | 9.90 |
| Cys | + | + | + | + | 9.27 | + |
| Val | 4.26 | 2.54 | 1.91 | 4.28 | 2.18 | + |
| Met | + | + | + | + | + | + |
| Ile | 3.30 | 2.42 | 1.41 | 0.78 | 0.71 | 1.61 |
| Leu | 5.14 | 3.57 | 1.94 | 1.59 | 1.42 | 2.34 |
| Tyr | 1.51 | 1.15 | + | + | + | 0.78 |
| Phe | 3.47 | 3.32 | 2.13 | 1.44 | 1.35 | 2.33 |
| His | 7.07 | 9.96 | 9.02 | 4.80 | 6.56 | 3.93 |
| γ-ABA | 10.29 | 13.20 | 15.24 | 15.18 | 13.36 | 17.32 |
| Lys | + | + | + | + | + | + |
| Arg | 68.70 | 84.74 | 78.48 | 30.83 | 29.73 | 39.64 |
| Orn | + | + | + | + | + | + |
| β-Ala | + | + | + | + | + | + |
| α-ABA | | | | | + | + |
| Try | + | + | + | + | + | + |
| OH-Pro | + | + | + | + | + | + |
| Sum | 207.22 | 217.54 | 179.00 | 128.24 | 116.93 | 120.86 |

Ø = normally treated
N = treated with N fertilizer
K = treated with K fertilizer

state to full maturity. The proline content increased 25–50 fold. The greatest arginine and proline contents were found in the Rizlingszilváni must. The concentration of arginine increased to 100 mg per 100 $cm^3$ and amounted to about 60% of the total free amino acid concentration (Figs. 1–2).

The accumulation of proline is also characteristic of the grape variety and the Rizlingszilváni is here also in the first place, reaching 46 mg per 100 $cm^3$ (Table 3). The relation between the proline content and the change of the

sugar–acid ratio during the maturation process shows that there is a positive correlation between the two variables, and this is significant at the P = 99% probabity level (Fig. 3).

As the quality and quantity of a vintage are determined by several factors: the climate of the preceeding year, the circumstances of the period of dormancy, and decisively the given growth season, in the evaluation of data of the amino acid analysis the investigation of the climatic factor cannot be neglected.

Table 2

*Change of composition of free amino acids in the berries of untreated Rizlingszilváni during maturation in 1977*
(mg per 100 $cm^3$)

| Amino acids | Date of sampling | | | | | |
|---|---|---|---|---|---|---|
| | 14 July | 26 July | 10 August | 24 August | 6 September | 19 September |
| Asp | 2.07 | 2.90 | 2.34 | 3.00 | 3.46 | 3.39 |
| Thr | 10.30 | 18.90 | 20.86 | 7.17 | 14.20 | 11.62 |
| Ser | | | | 17.22 | 0.06 | 0.24 |
| Glu | 12.00 | 12.50 | 6.52 | 7.30 | 10.17 | 16.01 |
| Pro | + | 2.60 | 4.32 | 9.88 | 27.44 | 25.48 |
| Gly | + | 0.39 | + | + | + | + |
| α-Ala | 3.00 | 2.60 | 4.38 | 6.55 | 15.46 | 7.63 |
| Cys | — | — | — | — | — | — |
| Val | 1.30 | 2.81 | 0.47 | 0.81 | 1.27 | 1.43 |
| Met | 0.65 | 0.37 | 0.36 | + | 0.58 | + |
| Ile | 0.43 | 0.54 | 0.69 | 0.42 | 0.61 | 0.76 |
| Leu | 0.40 | 0.50 | 0.94 | 0.70 | 1.08 | 1.21 |
| Tyr | + | 0.39 | 0.72 | 0.51 | 0.84 | 1.13 |
| Phe | 1.06 | 1.11 | 1.01 | 1.05 | 1.51 | 2.11 |
| His | 1.62 | 1.05 | 2.04 | 2.16 | 3.17 | 2.05 |
| γ-ABA | 1.18 | 0.65 | 1.20 | 1.57 | 2.27 | 4.34 |
| Lys | + | 0.85 | + | + | + | + |
| Arg | 22.57 | 42.20 | 44.60 | 69.00 | 82.65 | 79.51 |
| Orn | 0.50 | 0.61 | 0.64 | 0.77 | 0.30 | 0.42 |
| β-Ala | + | + | — | — | + | + |
| α-ABA | — | — | — | — | + | + |
| Tyr | + | + | + | + | 1.50 | 0.67 |
| OH-Pro | + | + | + | + | + | + |
| Sum | 57.08 | 90.97 | 91.09 | 128.11 | 174.57 | 164.00 |

In the years 1976–1978 climatic conditions were unfavourable, frost damages of early autumn and late spring occurred and the summer was droughty (Tables 4–5). The greatest rainfall was in 1976. In consequence of the wide-spread rains in September there was a danger of rot, and the vintage was performed earlier.

In 1977 the development of the grape was quicker because of the warm weather at the end of March and in June, and grape varieties of late ripening

reached maturity earlier. The greater amount of heat and less rainfall influenced positively the formation of sugar in the berries and even the development of the acidity. The vintage was weakest in 1978, when the sum of effective temperature of the growing season was less by 100 and 200 °C, respectively, than the average values of the previous years (Table 4).

Table 3

*Change of composition of free amino acids in the berries of untreated Rizlingszilváni during maturation in 1978*
(mg per 100 cm³)

| Amino acids | Date of sampling | | | | | | |
|---|---|---|---|---|---|---|---|
| | 24 July | 8 August | 21 August | 4 September | 19 September | 2 October | 17 October |
| Asp | 4.82 | 3.09 | 10.59 | 9.60 | 6.29 | 3.25 | 3.11 |
| Thr | 16.93 | 17.85 | 15.23 | 18.63 | 14.28 | 15.73 | 13.72 |
| Ser | 14.30 | 16.10 | 17.38 | 15.31 | 14.84 | 15.54 | 15.20 |
| Glu | 10.86 | 8.30 | 10.82 | 13.06 | 19.87 | 22.19 | 19.62 |
| Pro | 1.17 | 1.27 | 1.62 | 14.79 | 15.60 | 42.57 | 46.65 |
| Gly | 0.35 | 0.10 | 0.13 | 0.40 | 0.45 | 0.57 | 0.38 |
| α-Ala | 3.47 | 1.24 | 2.78 | 8.25 | 9.15 | 13.12 | 8.34 |
| Cys | — | — | — | — | — | — | — |
| Val | 1.26 | 0.46 | 0.83 | 1.87 | 1.85 | 6.36 | 6.66 |
| Met | 0.16 | 0.58 | 0.38 | 0.50 | 0.63 | 1.76 | 1.53 |
| Ile | 0.32 | 0.38 | 0.57 | 1.28 | 1.72 | 5.29 | 5.40 |
| Leu | 0.64 | 0.71 | 1.45 | 1.71 | 2.21 | 6.65 | 6.13 |
| Tyr | 0.59 | 0.64 | 1.64 | 1.97 | 1.91 | 2.14 | 1.43 |
| Phe | 1.60 | 0.54 | 1.41 | 3.37 | 3.56 | 6.81 | 4.88 |
| His | 1.41 | 1.43 | 3.10 | 3.26 | 2.05 | 2.58 | 3.19 |
| γ-ABA | + | 0.34 | 1.94 | 2.70 | 2.65 | 5.07 | 8.49 |
| Lys | 0.28 | 0.38 | 0.51 | 0.52 | + | 0.33 | + |
| Arg | 6.97 | 11.57 | 28.95 | 46.25 | 57.00 | 75.45 | 62.50 |
| Orn | + | + | + | + | + | + | + |
| β-Ala | + | — | — | — | + | + | + |
| α-ABA | — | — | — | — | + | + | + |
| Try | + | + | + | + | + | + | + |
| OH-Pro | + | + | + | + | + | + | + |
| Sum | 64.63 | 64.98 | 99.33 | 143.67 | 153.06 | 225.31 | 207.23 |

In 1977 the amount of arginine in the must was greater than in 1978.

The change of the proline content was influenced rather by the annual amount of rainfall and the rainfall during the growing season and the intensity of light, than the rise or fall of temperature. While the amount of rainfall showed a negative relationship, the intensity of light showed a positive relationship with the development of proline content (Tables 5–6).

No correlation was found between the proline content and the use of nutrients (Table 6) but there is a positive correlation between the treatment with nutrients and the arginine content (Figs. 1–2). The treatment with potassium resulted in a lower arginine content of the must.

Organoleptic analysis of the Rizlingszilváni wines from the years 1977 and 1978 showed that the best results, the highest scores were reached in both years by wines treated with potassium fertilizer. The lowest scores were given to wines which were treated with nitrogen fertilizer (Table 7).

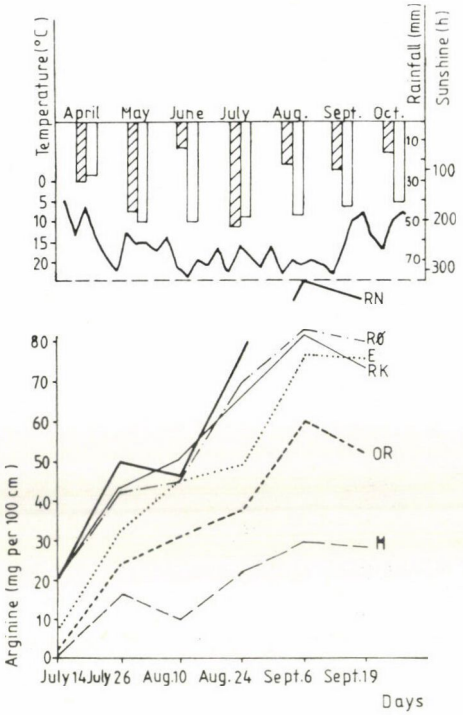


Fig. 1. Changes in the arginine content of must samples of differently fertilized grape varieties during maturation, giving information on the temperature in °C (—), rainfall in mm (▨), and sunshine in h (□) in 1977. Abbreviations: RØ=Rizlingszilváni, normally fertilized, RN=Rizlingszilváni, treated with surplus N-fertilizer, RK=Rizlingszilváni, treated with surplus K-fertilizer, E=Ezerjó, H=Hárslevelű, OR=Olaszrizling

## 3. Conclusions

With the advancement of maturation, especially in the last phase of maturation of the grape, processes of dehydration begin to predominate, in the course of which the accumulation of sugar, the growth of the respiratory quotient, the deamination of amino acids take place. As the result of the latter processes ammonia and α-ketoglutaric acid accumulate. This latter compound is utilized in biosynthesis, because the intensive formation of sugar prevents the oxidation of proline (STEWART et al. 1966).

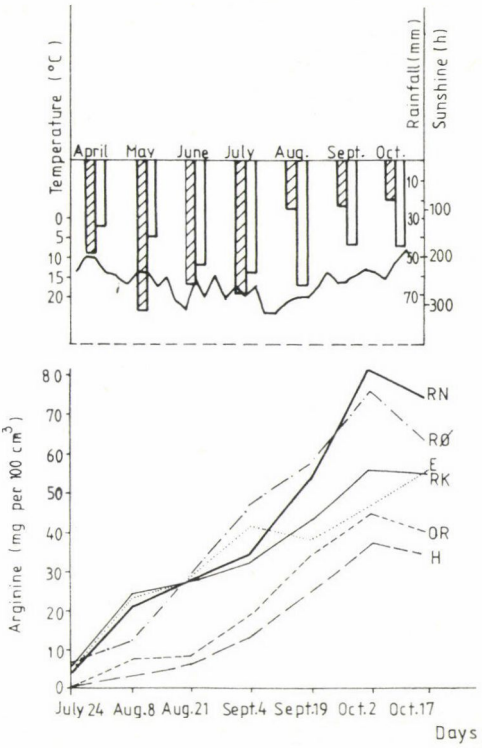


Fig. 2. Changes in the arginine content of must samples of differently fertilized grape varieties during maturation, giving information on the temperature in °C (—), rainfall in mm (▨) and sunshine in h (□) in 1978. Abbreviations: RØ-Rizlingszilváni, normally fertilized, RN=Rizlingszilváni, treated with surplus N-fertilizer, RK=Rizlingszilváni, treated with surplus K-fertilizer, E=Ezerjó, H=Hárslevelű, OR=Olaszrizling

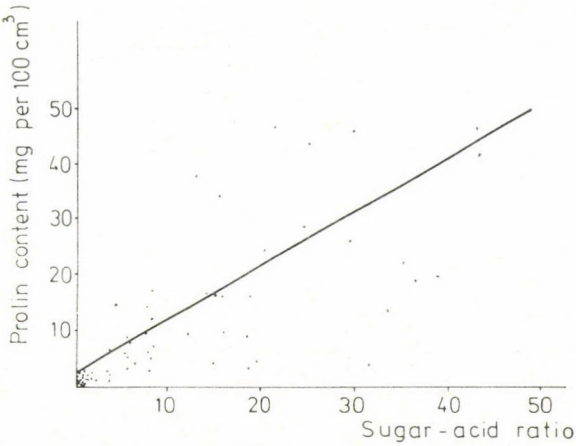


Fig. 3. Line of regression ($y$) showing relationship between the proline content of musts and the sugar–acid ratio. $y = 0.68\,x + 2.73$, $r = 0.74$

Table 4

*Growing season of cultivated grape varieties and some climatic features in Szigetcsép*

| Observed factors | 1976 | 1977 | 1978 |
|---|---|---|---|
| Beginning of growing season | 7 April | 17 April | 12 April |
| Date of last significant spring frost | 1 May | 21 April | 13 May |
| End of growing season | 26 October | 10 October | 12 October |
| Date of first significant autumn frost | — | 28 September | — |
| Duration of growing season (days) | 203 | 177 | 193 |
| Modified growing season because of damage by frost (days) | 179 | 160 | 162 |
| Sum of effective temperature during growing season (°C) | 3502.4 | 3206.3 | 3141.5 |
| Amount of effective heat during growing season modified by frost damage (°C) | 1440.2 | 1379.2 | 1244.8 |
| Yearly average of temperature (°C) | 10.0 | 11.1 | 10.1 |
| Average temperature during growing season (°C) | 17.3 | 16.9 | 15.9 |
| Average temperature during growing season modified by frost damage (°C) | 18.0 | 18.6 | 17.2 |
| Total yearly amount of rainfall (mm) | 617.6 | 446.9 | 449.0 |
| Rainfall during growing season (mm) | 424.5 | 195.4 | 283.0 |
| Rainfall during growing season modified by frost damage (mm) | 359.7 | 186.2 | 222.8 |

Experiments made with other test plants support also the conclusion that when the amount of the carbohydrates decreases in the leaves during translocation or respiration, the proline is metabolized. The conversion of proline to glutamic acid produces 2 molecules of reduced nicotinamide adenine dinucleotide phosphate and in this way the proline is a potential source of energy and reduction for the plants under unfavourable climatic conditions (STEWART et al., 1966; STEWART & LEE, 1974). It can be explained with this important physiological function that proline accumulates under stress effects as high intensity of light and minimal rainfall (COOMBE & MONK, 1979). The decrease of the arginine level and the increase of the proline content — as it is seen in the last sample — shows, that in the case of stress (in 1977 a sudden fall in the temperature, in 1978 the lack of rainfall) proline may be synthesized even in the grape through the ornithine cycle.

Table 5

*Climatic data in Szigetcsép*

| Month | Monthly and yearly averages of air temperature (°C) | | | | Monthly and yearly sum of rainfall (mm) | | | | Monthly and yearly sum of sunshine (h) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1976 | 1977 | 1978 | average of three years | 1976 | 1977 | 1978 | average of three years | 1976 | 1977 | 1978 | average of three years |
| January | 0.8 | —0.1 | 0.1 | 0.2 | 25.0 | 41.0 | 22.0 | 29.3 | 56.0 | 45.0 | 63.0 | 54.7 |
| February | —0.1 | 4.2 | 0.4 | 1.5 | 3.0 | 53.0 | 28.0 | 28.0 | 93.0 | 88.0 | 57.0 | 79.3 |
| March | 2.8 | 9.0 | 7.2 | 6.3 | 28.0 | 58.0 | 27.0 | 37.7 | 156.0 | 165.0 | 154.0 | 158.3 |
| April | 12.4 | 9.7 | 10.4 | 10.8 | 65.0 | 30.0 | 48.0 | 47.7 | 169.0 | 108.0 | 138.0 | 138.3 |
| May | 17.1 | 17.1 | 14.4 | 16.2 | 20.0 | 45.0 | 77.0 | 47.3 | 199.0 | 203.0 | 158.0 | 186.7 |
| June | 20.4 | 21.2 | 18.7 | 20.1 | 30.0 | 13.0 | 64.0 | 35.7 | 207.0 | 204.0 | 217.0 | 209.3 |
| July | 23.1 | 20.8 | 20.2 | 21.4 | 105.0 | 54.0 | 69.0 | 76.0 | 211.0 | 197.0 | 233.0 | 213.7 |
| August | 19.2 | 20.1 | 20.1 | 19.8 | 42.0 | 21.0 | 25.0 | 29.3 | 193.0 | 194.0 | 248.0 | 211.7 |
| September | 15.3 | 14.9 | 15.7 | 15.3 | 92.0 | 25.0 | 24.0 | 47.0 | 122.0 | 176.0 | 176.0 | 158.0 |
| October | 11.6 | 12.0 | 11.6 | 11.7 | 80.0 | 16.0 | 21.0 | 39.0 | 99.0 | 165.0 | 180.0 | 148.0 |
| November | 7.0 | 5.7 | 2.2 | 5.0 | 30.0 | 61.0 | 16.0 | 35.7 | 53.0 | 81.0 | 16.0 | 50.0 |
| December | 0.1 | —1.2 | 0.8 | —0.1 | 99.0 | 29.0 | 28.0 | 52.0 | 37.0 | 32.0 | 37.0 | 35.3 |
| Sum | — | — | — | — | 619.0 | 446.0 | 449.0 | 509.6 | 1595.0 | 1658.0 | 1677.0 | 1643.3 |
| Average | 10.8 | 11.1 | 10.1 | 10.7 | — | — | — | — | — | — | — | — |

Table 6

*Change of proline content in the musts of grape varieties untreated and treated with fertilizers of large quantity N and K*
(mg per 100 $cm^3$)

| Varieties / Date of picking | Untreated | | | | Treated with high quantity fertilizers | |
|---|---|---|---|---|---|---|
| | Ezerjó | Hárslevelű | Olasz-rizling | Rizling-szilváni | Rizlingszilváni N | Rizlingszilváni K |
| 1976 | | | | | | |
| July 27 | + | + | + | + | + | + |
| August 24 | 4.49 | + | + | + | 12.20 | 10.25 |
| September 21 | 14.50 | 8.73 | + | 16.38 | 18.00 | 17.13 |
| 1977 | | | | | | |
| July 14 | + | 0.83 | + | + | + | + |
| July 26 | 0.42 | + | 1.26 | 2.60 | 1.08 | 1.63 |
| August 10 | 6.45 | 1.45 | 1.38 | 4.32 | 5.06 | 5.22 |
| August 24 | 12.40 | 2.11 | 1.53 | 9.88 | 16.70 | 6.53 |
| September 6 | 34.56 | 3.30 | 4.56 | 27.44 | 28.68 | 16.38 |
| September 19 | 24.70 | 3.51 | 4.14 | 25.48 | 26.01 | 19.16 |
| 1978 | | | | | | |
| July 24 | 0.34 | 0.43 | 0.51 | 1.17 | 0.42 | 0.78 |
| August 8 | 1.20 | 1.31 | 0.98 | 1.27 | 0.68 | 2.13 |
| August 21 | 2.63 | 1.64 | 2.30 | 1.62 | 4.30 | 2.30 |
| September 4 | 14.38 | 2.06 | 1.00 | 14.79 | 8.87 | 8.21 |
| September 19 | 17.09 | 5.75 | 2.96 | 15.40 | 16.43 | 16.43 |
| October 2 | 37.88 | 7.44 | 9.81 | 42.57 | 27.74 | 43.71 |
| October 17 | 46.65 | 9.31 | 4.90 | 46.65 | 22.28 | 20.05 |

Unfavourable environmental conditions have an influence on the assimilation of the ammonium and nitrate ions. The effect of the vintages, which may be characterized with different ecological conditions is shown also in the results. As it is well known, the activity of nitrate reductase may be induced by the substrate. In 1978 the abundant rainfall in the period from April to June, which amounted to 60% of the precipitation of the year, hindered pollination and fertilization, it promoted the washing down of the nitrates. Presumably the activity of the nitrate reductase decreased and therefore there are no significant differences in the free amino acid and the arginine content between treated and untreated Rizlingszilváni samples. Comparing the results of the three vintages it may be stated, that the effect of treatment with nitrogen fertilizer is not equivocal in every year on the titratable acidity (tartaric acid), pH and refractive index (%) values.

The role of the free amino acids in the establishment of the taste, flavour of the wines is controversial and unexplained (DI STEFANO, 1979; OUGH & BELL, 1980). One part of the free amino acids exerts favourable influence on the organoleptic properties of the musts and wines. To the proline, being present in the greatest amount in the must of the grape Rizlingszilváni, a positive

Table 7

*Analytical data of musts and wines in the case of normally treated (Ø) Rizlingszilváni and treated with excessive amounts of N and K fertilizers*

| Test | Ø | N | K | Ø | N | K |
|---|---|---|---|---|---|---|
| | 1977 | | | 1978 | | |
| | Results of must analysis | | | | | |
| Total free amino acids (mg per 100 $cm^3$) | 164.0 | 216.73 | 207.2 | 207.2 | 171.6 | 157.7 |
| Ariginine (mg per 100 $cm^3$) | 79.5 | 110.20 | 73.1 | 62.5 | 68.6 | 53.7 |
| Proline (mg per 100 $cm^3$) | — | — | — | 60.0 | 100.0 | 81.0 |
| Refractive index (%) | 18.9 | 18.1 | 18.6 | 20.1 | 17.3 | 18.3 |
| Titratable acidity (g $dm^{-3}$) | 4.8 | 4.7 | 7.0 | 5.8 | 6.1 | 5.6 |
| Tartaric acid (g $dm^{-3}$) | 142.5 | 140.0 | 102.5 | 82.8 | 131.7 | 93.8 |
| Weight of cluster (g) | 224.86 | 276.9 | 249.4 | 212.8 | 204.6 | 205.2 |
| Weight of berry (g) | 2.17 | 2.45 | 1.91 | 1.74 | 1.70 | 1.79 |
| Flowering (%) | + | + | + | 84.13 | 71.43 | 79.69 |
| Test | Results of wine analysis | | | | | |
| Alcohol ($M^0$) | 10.2 | 9.8 | 10.0 | 11.0 | 7.2 | 11.2 |
| Titratable acidity (g $dm^{-3}$) | 7.0 | 6.1 | 6.5 | 6.4 | 6.8 | 7.2 |
| Total extract (g $dm^{-3}$) | 17.9 | 16.3 | 19.2 | 18.2 | 18.2 | 26.1 |
| Extract less the sugar (g $dm^{-3}$) | 12.6 | 11.8 | 14.4 | 5.2 | 10.9 | 13.5 |
| Ash (g $dm^{-3}$) | 1.5 | 1.5 | 1.7 | 1.4 | 1.5 | 1.6 |
| Average of scores of organoleptic examination | 16.95 | 16.33 | 17.53 | 16.68 | 16.49 | 17.08 |
| Average of scores (%) | 84.75 | 81.65 | 87.65 | 83.40 | 82.45 | 85.40 |

effect may be contributed from the point of view of grape quality (OUGH & ALLEY, 1970). This is supported by the positive correlation of the proline content and the sugar–acid ratio (Fig. 3) and by the observation that in the wines fermented from musts the concentration of proline is not or only slightly altered in contrast with arginine concentration (53.0–59.5 mg per 100 $cm^3$). This may be attributed to the fact, that yeasts do not assimilate proline and its presence influences in any case the quality of the wines (KLIEWER, 1968).

The accumulation of other amino acids in the musts may have worse effect on the organoleptic properties of the wines. Wines, which were fermented from musts containing high amounts of arginine, got the lowest scores (Table 7).

To decide, whether the arginine itself, or a product of it is the cause of the deterioration of the organoleptic quality of the wines, the free amino acid content of the wines was also investigated. It was found, that the concentration of arginine may be explained on the one hand by the fact, that yeasts assimilated a great part of the arginine and on the other, secondary products may be formed from arginine which impair the organoleptic properties of the

wines. Other authors also confirmed this view who found a negative correlation between some volatile components and the arginine content of the grape (CORDNER et al., 1978).

The investigation of the components determining the hedonic value of wine showed, that a greater amino acid and arginine content, and a high tartaric acid content influence adversely the quality. It can be stated that excessive amounts of potassium fertilizer exerts a positive effect on the quality of wine and excessive amounts of nitrogen fertilizer have a negative effect. It would be perhaps useful to determine the optimal fertilizer doses for all grape varieties.

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# COMPARATIVE INVESTIGATIONS INTO THE DETERMINATION OF PROTEIN BY THE KJELDAHL METHOD AND NIR TECHNIQUE

L. HORVÁTH,[a] K. NORRIS[b] and M. HORVÁTH-MOSONYI[c]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] USDA-ARS, Beltsville Agricultural Research Center, Instrumentation Research Laboratory, Beltsville, Maryland 20705, USA
[c] Faculty of Advanced Paramedical Training, Institute for Postgraduate Medical Education, Department of Dietetics, H-1085 Budapest, Makarenko u. 24. Hungary

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For this comparative investigation, 30 samples were prepared by mixing wheat flour with phenylalanine, wheat gluten and cellulose in known amounts.

Near-infrared reflectance (R) spectra were recorded and transformed to log (1/R). The ratio of second derivatives of log (1/R) spectra was correlated with compositional data. The NIR technique gave a correlation of 0.995 to Kjeldahl protein at 2177.6 nm and a correlation of 0.996 to calculated protein at 2062.4 nm.

At a numerator wavelength of 2177.6 nm, NIR provided a correlation of 0.995 to Kjeldahl protein and at 2062.4 nm a correlation of 0.996 to calculated protein. Thus, the NIR technique provided adequate discrimination for protein in spite of large variation in non-protein nitrogen.

A correlation of 0.999 was obtained between phenylalanine and NIR data using a second derivative ratio with the numerator wavelength at 1140.8 nm. For cellulose, the correlation coefficient was 0.999 using a second derivative with the numerator wavelength at 2336.0 nm. Each of the numerator wavelengths giving the high correlations coincided with the location of an absorption band for the respective component.

**Keywords:** Protein determination by NIR technique, wheat-flour protein determination, comparison of Kjeldahl method–NIR technique

The most accepted and wide-spread method for determining protein content of foods in traditional food analysis is the Kjeldahl method. This method has two well-known sources of error:

presence of non-protein nitrogen in the sample;

variability of the nitrogen to protein factor.

Efforts have been made for a long time to elaborate a method for determining protein, specific to protein, and able to eliminate the error which is inherent in the Kjeldahl method because the Kjeldahl method cannot take into account that the N content of different proteins is different.

One of the characteristic reactions to detect protein is the biuret reaction which is used also for quantitative determination of protein (BEISENHERZ et al., 1953; LÁSZTITY & TÖRLEY, 1971). The basis for this reaction is the formation of a lilac-blue complex between peptid binding of protein or polypeptids and $Cu^{2+}$ ions in the presence of NaOH and tartrates. The biuret reaction is not

very sensitive, but very characteristic of peptid binding (SCHORMÜLLER, 1967; SZEKERES, 1967).

Attempts have been made to determine the protein content of foods by the NIR technique, as well, and the Kjeldahl method has been used for the calibration (KAFFKA et al., 1982a, b, c). In these experiments, because of the above errors of the Kjeldahl method, one of the most important advantages of the NIR technique could not be achieved i.e. great accuracy in predicting protein content.

In the course of our preliminary investigations, attempts were made to get more information on using the Kjeldahl method for the calibration of the NIR technique and obtaining a comparison between the two methods.

## 1. Materials and methods

### *1.1. Preparation of the samples*

For the comparison of the NIR and Kjeldahl methods in the analysis of foods, wheat flour was chosen as model material and different mixtures with other materials were made:

- wheat flour and a free amino acid at different levels; varying Kjeldahl nitrogen but no variation in protein,
- wheat flour and flour protein (gluten); varying Kjeldahl nitrogen and protein,
- wheat flour and cellulose powder; variation neither in Kjeldahl nitrogen nor in protein.

Wheat flour was purchased on the market (Hungarian wheat flour, BL 55, of 0.55% ash content).

Phenylalanine was used as free amino acid to raise the total N but not to raise protein N because this is one of the amino acids occurring in the flour in greatest concentration, 0.55 g per 100 g (SOUCI et al., 1979) and because as a powder it could be easier homogenized than e.g. leucine.

Gluten was made at the Pastry Plant, Demecser.

Cellulose powder was a special preparation for chromatographic use (MN 300).

Altogether 30 samples were made in three groups, with the three materials mentioned above, of the following compositions:

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| flour | (%) | 99 | 98 | 97 | 96 | 95 | 94 | 93 | 92 | 91 | 90 |
| additive | (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |

Protein determination of the 30 samples was performed by the traditional Kjeldahl method, computing protein percent as N (%) $\times$ 5.7 as it is generally

used for wheat flour. (A.O.A.C., 1980) The data obtained by the Kjeldahl method used for comparison are corrected data, determined from the regression line of the measured data.

*1.2. NIR spectrophotometer*

The reflectance spectra of wheat bran samples were recorded with a multipurpose computerized spectrophotometer using a Cary Model 14 prism-grating monochromator built at USDA-BARC-Instrumentation Research Laboratory, Beltsville, USA.

The instrument is operated in a single-beam mode with slits of 2 mm giving an effective bandpass of 7 nm. Ceramic material was used as a reference standard. A new spectrum of the reference standard was recorded once each hour to minimize the influence of long-term drift. The measuring geometry was 0° per 45°. The wavelength range from 1000 to 2638.4 nm was scanned with a speed of 10 nm per second. To measure a reflectance spectrum requires about 164 s. Reflectance (R) data measured with four lead-sulfide detectors were collected every 0.2 nm, with 256 readings per point. We got 8192 reflectance points for the whole wavelength range. For data processing, the 8192 point spectral curves were smoothed by a running average of 21 points and shrunk to 1024 point curves by using the average of each 8 points.

Compressed curves for all samples were transformed to log (1/R) because this function gives a linear correlation with the concentration of a given measured component. Thereafter, the data were recorded on magnetic tape for further processing on a Hewlett—Packard 1000F computer. After recording measured chemical data, the second derivative of the log (1/R) was produced. The studies of NORRIS (1983) and KAFFKA and co-workers (1982a, b, c) indicated that using the second derivative form of log (1/R) gives better results than log (1/R). The second derivative calculation was incorporated into the computer program for the linear regression analysis. The regression determined the optimum wavelengths, the constants for the calibration equation, the correlation coefficient, and the standard error of calibration for each constituent.

The definition of standard error of calibration used in the regression analysis is

$$\sqrt{\frac{\Sigma\,(Q_{si}-Q_{ci})^2}{n-p-1}}$$

where:

$Q_s$: component concentration determined by chemical analysis (mass %)
$Q_c$: component concentration calculated by the regression equation (mass %)
$n$: number of samples,
$p$: number of independent variables.

The reflection measurements described were carried out in an air conditioned laboratory at a temperature of 22 °C.

## 2. Results

Compositional data of the 30 wheat flour samples used in the regression analysis are summarized in Table 1.

Compositional data of 30 wheat flour samples given in Table 1 are not independent from each other. The correlation coefficients between the compositional data can be seen in Table 2.

Correlations between the compositional data of 30 wheat flour samples are important because they are the limits of correlations between the NIR predicted values in further regression analysis. The primary correlations between the compositional data and the NIR predicted values for the same constituents must be high; the secondary correlations between corresponding values of different constituents should be low.

The log (1/R) spectrum and the second-derivative curve of wheat flour, wheat gluten, phenylalanine and cellulose are presented in Figs. 1—4.

Table 1

*Compositional data of the 30 wheat flour samples*
(mass %)

| Sample No. | Protein (Kjeldahl) | Protein (calculated) | L-phenyl-alanine | Cellulose |
|---|---|---|---|---|
| 1 | 10.90 | 10.46 | 1.54 | 2.23 |
| 2 | 11.21 | 10.34 | 2.54 | 2.21 |
| 3 | 11.55 | 10.24 | 3.53 | 2.18 |
| 4 | 11.88 | 10.13 | 4.53 | 2.16 |
| 5 | 12.22 | 10.03 | 5.52 | 2.14 |
| 6 | 12.55 | 9.92 | 6.52 | 2.12 |
| 7 | 12.88 | 9.82 | 7.51 | 2.09 |
| 8 | 13.21 | 9.71 | 8.51 | 2.07 |
| 9 | 13.54 | 9.61 | 9.50 | 2.05 |
| 10 | 13.87 | 9.50 | 10.50 | 2.03 |
| 11 | 10.55 | 10.46 | 0.54 | 3.23 |
| 12 | 10.45 | 10.34 | 0.54 | 4.21 |
| 13 | 10.36 | 10.24 | 0.53 | 5.18 |
| 14 | 10.26 | 10.13 | 0.53 | 6.16 |
| 15 | 10.17 | 10.03 | 0.52 | 7.14 |
| 16 | 10.08 | 9.92 | 0.52 | 8.12 |
| 17 | 9.99 | 9.82 | 0.51 | 9.09 |
| 18 | 9.89 | 9.71 | 0.51 | 10.07 |
| 19 | 9.80 | 9.61 | 0.50 | 11.05 |
| 20 | 9.70 | 9.50 | 0.50 | 12.03 |
| 21 | 11.99 | 11.94 | 0.54 | 2.23 |
| 22 | 12.55 | 12.51 | 0.54 | 2.21 |
| 23 | 13.10 | 13.08 | 0.53 | 2.18 |
| 24 | 13.65 | 13.60 | 0.53 | 2.16 |
| 25 | 14.20 | 14.23 | 0.52 | 2.14 |
| 26 | 14.75 | 14.80 | 0.52 | 2.12 |
| 27 | 15.30 | 15.38 | 0.51 | 2.09 |
| 28 | 15.86 | 15.95 | 0.51 | 2.07 |
| 29 | 16.41 | 16.50 | 0.50 | 2.05 |
| 30 | 16.96 | 17.10 | 0.50 | 2.03 |

Table 2

*Correlation coefficients between the compositional data of the 30 wheat flour samples*

| Component No. | Protein (Kjeldahl) | Protein (calculated) | Phenylalanine | Cellulose |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 1 | 1.000 | 0.828 | 0.156 | —0.684 |
| 2 | 0.828 | 1.000 | —0.424 | —0.429 |
| 3 | 0.156 | —0.424 | 1.000 | —0.366 |
| 4 | —0.684 | —0.429 | —0.366 | 1.000 |

Results of single-term regression analysis, namely the characteristic wavelengths, constants, coefficients of equations and standard errors of calibration and correlation coefficients are summarized in Table 3.

In Table 3 the gap used in regression analysis is also given. The gap is the distance among the three wavelengths used for producing the second derivative. The results were determined for the average spectra of three replicas for each sample.

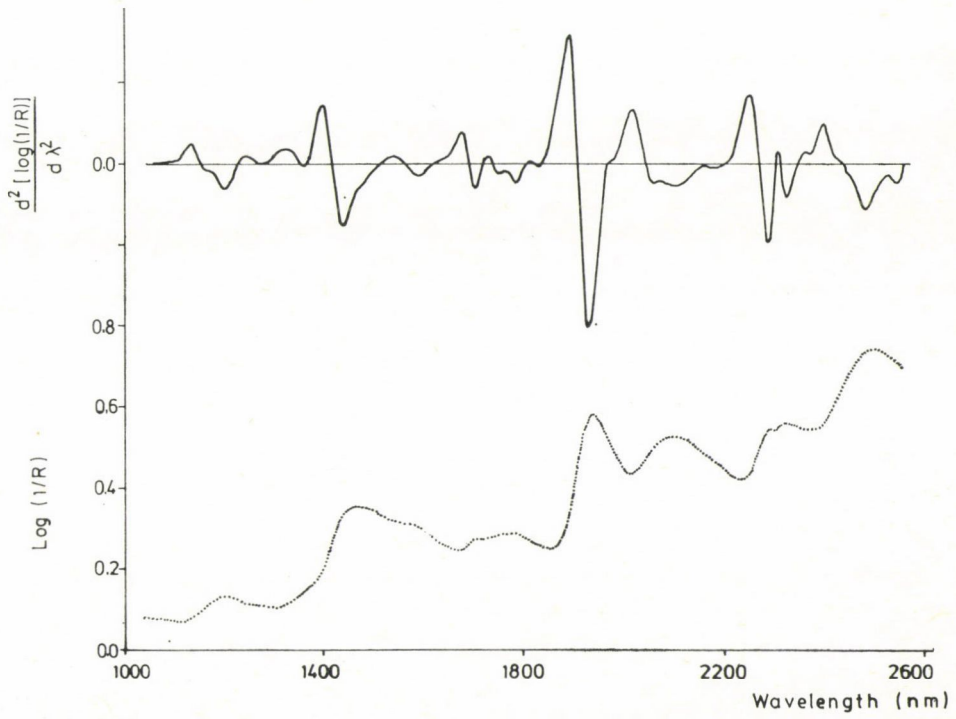


Fig. 1. The log (1/R) spectrum and the second derivative curve of wheat flour

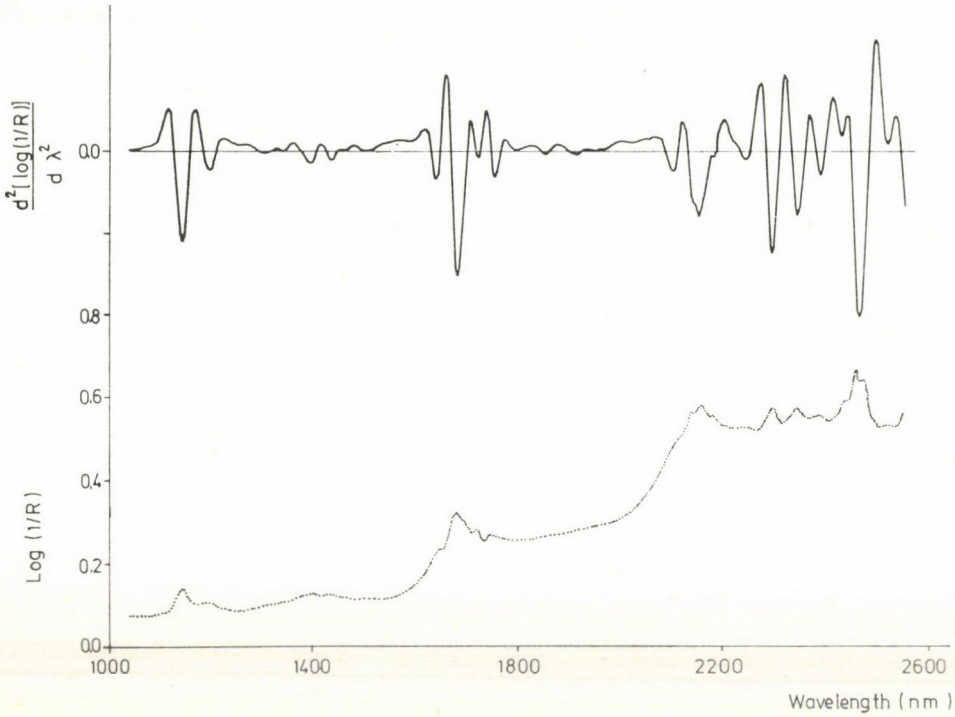


Fig. 2. The log (1/R) spectrum and the second derivative curve of phenylalanine

Table 3

*Summary of linear regression analyses relating compositional data and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 30 wheat flour samples*

| | Equation form $Q_i = K_{oi} + K_{1i}(V''_{\lambda_{1i}}/V''_{\lambda_{2i}})$ | | | |
|---|---|---|---|---|
| | Equation No. | | | |
| | I | II | III | IV |
| | Protein (Kjeldahl) | Protein (calculated) | Phenylalanine | Cellulose |
| $\lambda_1$ | 2177.6 | 2062.4 | 1140.8 | 2336 |
| $\lambda_2$ | 1166.4 | 1569.4 | 1472.0 | 1544 |
| $K_0$ | 11.026 | —10.093 | 7.9415 | —5.5690 |
| $K_1$ | 12.835 | 10.994 | 11.726 | —4.8533 |
| Gap at $\lambda_1$ | 24 | 19 | 13 | 30 |
| Gap at $\lambda_2$ | 23 | 21 | 15 | 30 |
| Sec | 0.21 | 0.13 | 0.32 | 0.17 |
| R | 0.995 | 0.996 | 0.995 | —0.999 |

Sec: standard error of calibration;
R: correlation coefficient
$\lambda_1$; $\lambda_2$: characteristic wavelengths
$K_0$; $K_1$: coefficients and constants

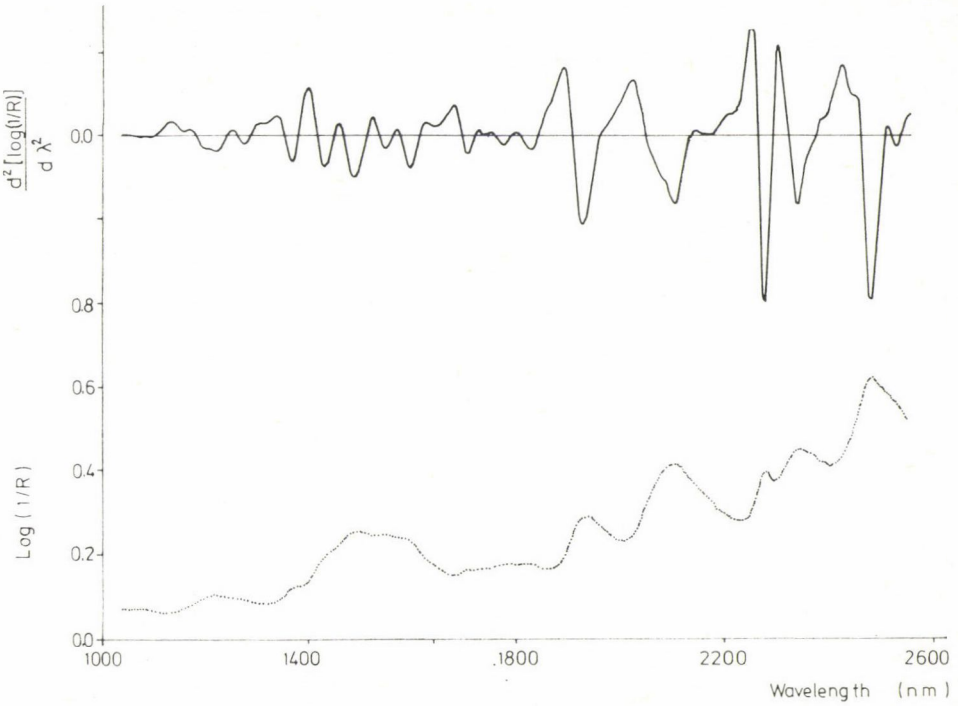


Fig. 3. The log (1/R) spectrum and the second derivative curve of cellulose

The relationships between compositional data given in Table 1 and predicted values determined by the equations presented in Table 3 for both values of protein, phenylalanine, and cellulose appear in Table 4 and in Figs. 5–8.

Table 4

*Correlation coefficients between the compositional data and predicted data using the equations of both forms of protein, phenylalanine and cellulose given in Table 3*

| Component No. | Equation No. I | II | III | IV |
|---|---|---|---|---|
| | Protein (Kjeldahl) | Protein (calculated) | Phenylalanine | Cellulose |
| | 1 | 2 | 3 | 4 |
| 1 | 0.995 | 0.839 | 0.152 | 0.668 |
| 2 | 0.817 | 0.999 | −0.426 | 0.408 |
| 3 | 0.169 | −0.404 | 0.992 | 0.377 |
| 4 | −0.680 | −0.449 | −0.363 | −0.999 |

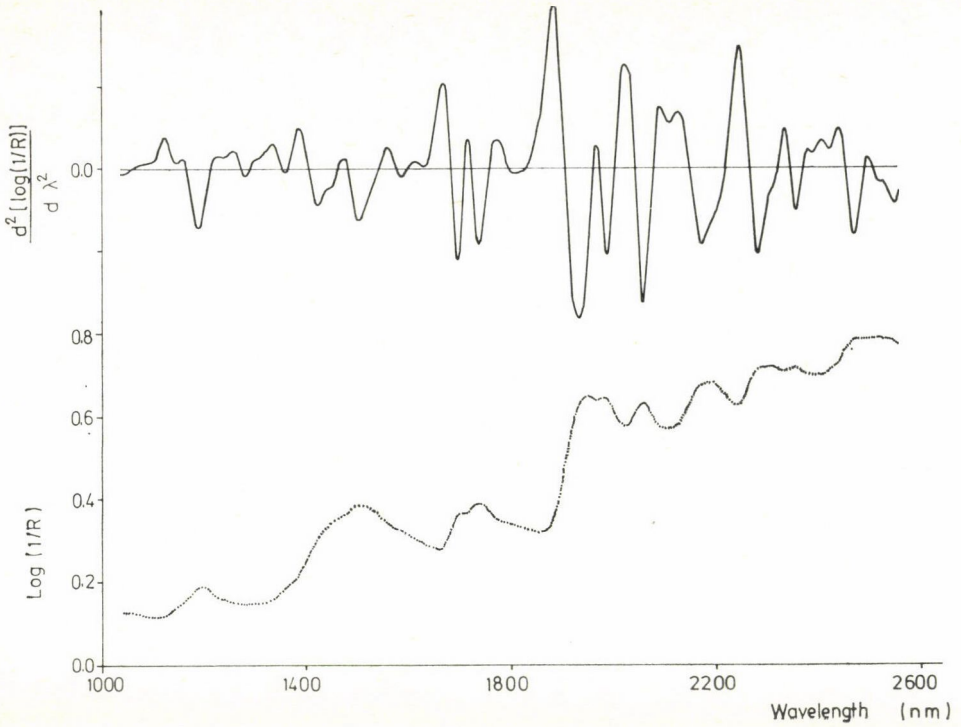


Fig. 4. The log (1/R) spectrum and the second derivative curve of wheat gluten

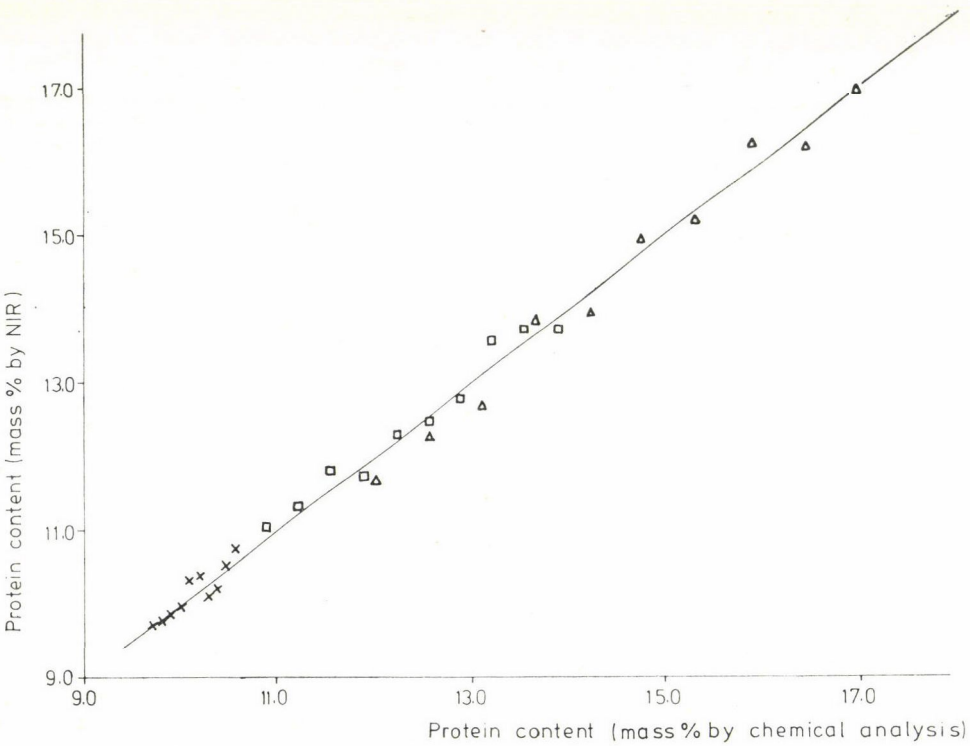


Fig. 5. Relationship between calculated protein content determined by Kjeldahl analysis and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths; □: samples with phenylalanine; x: samples with cellulose; △: samples with wheat gluten

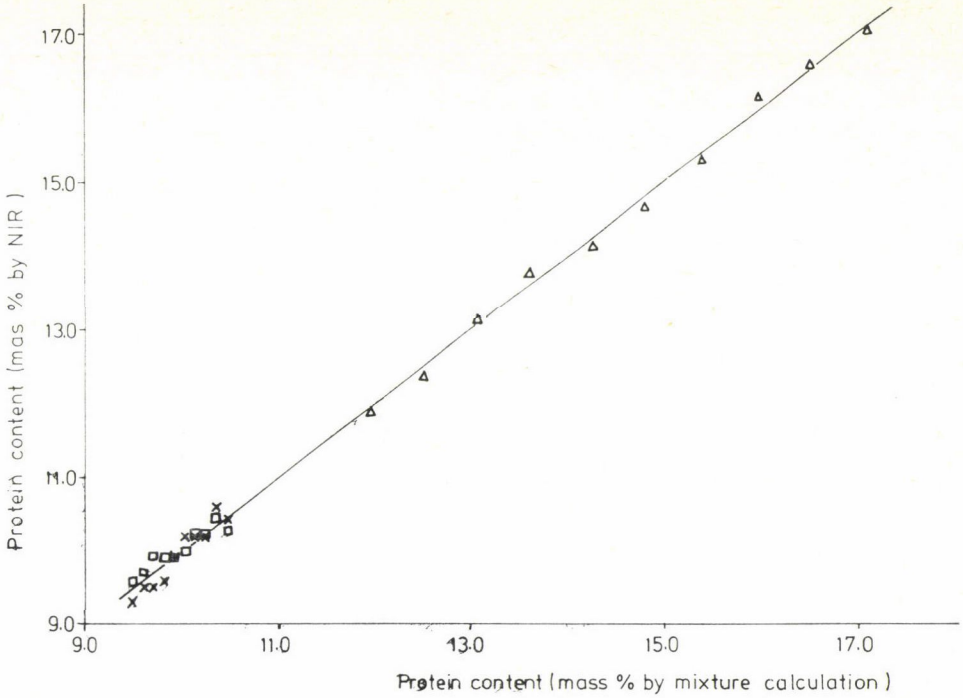


Fig. 6. Relationship between protein content calculated from the mixing ratio and predicted value from linear regression of the ratio of the second derivative of the log (1/R) curves at two wavelengths. □: samples with phenylalanine; x: samples with cellulose; △: samples with wheat gluten

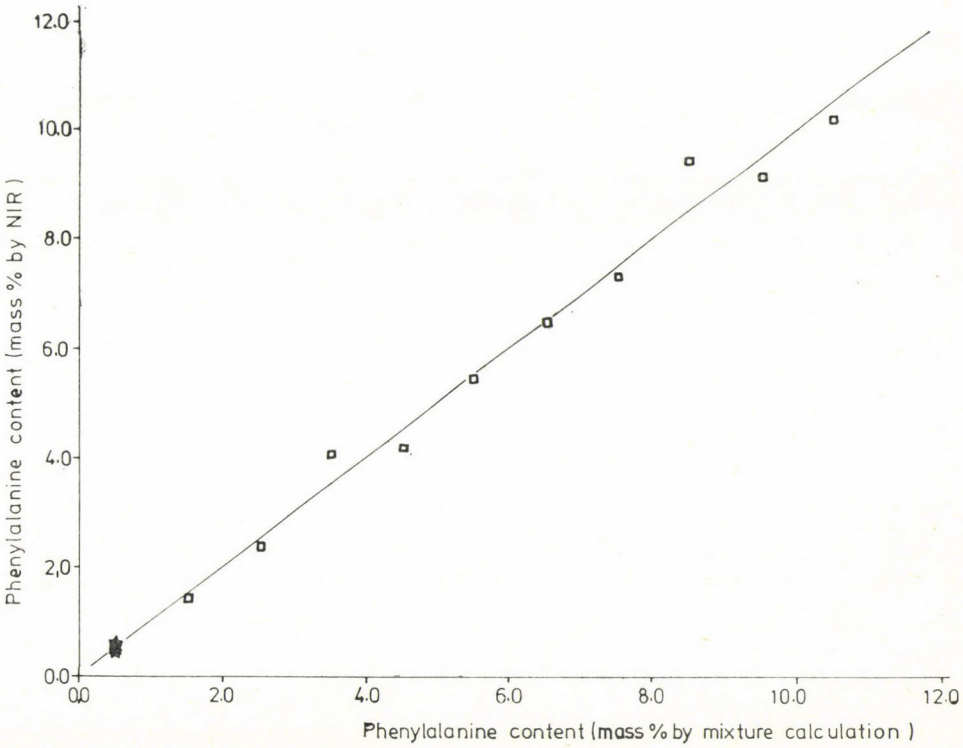


Fig. 7. Relationship between phenylalanine content calculated from the mixing ratio and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. □: samples with phenylalanine; x: samples with cellulose; △: samples with wheat gluten

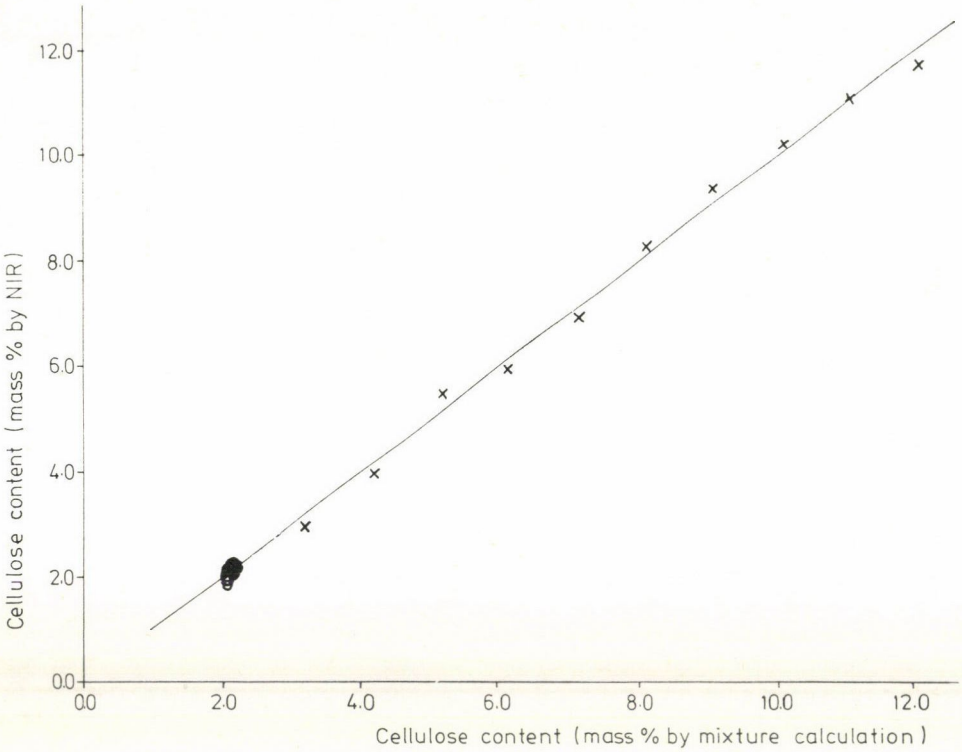


Fig. 8. Relationship between cellulose content calculated from the mixing ratio and predicted value from linear regression of the ratio of the second derivatives of the log (1/R) curves at two wavelengths. □: samples with phenylalanine; x: samples with cellulose △: samples with wheat gluten

## 3. Conclusions

The data of Table 1 show that by enriching the wheat flour with phenylalanine, cellulose and wheat gluten, the protein concentration increased from 9.5 to 17, the phenylalanine concentration from 0.5 to 10.5 and the cellulose concentration from 2 to 12 mass percent.

The correlation coefficients between components varied from 0.156 to 0.828. These correlations are low enough to permit independent evaluations between NIR data and each component.

Using spectral analysis in the near-infrared range, the primary aim in our study was to verify if it is possible to differentiate between the nitrogen and protein determinations. Data in Table 3 show the differences between the wavelengths that were found for the best calibrations. NORRIS (1983) has drawn attention to numerator wavelengths that are important in the identification of components. Examination of Fig. 4 shows that wheat gluten has

an absorption band at both 2062.4 nm and 2177.6 nm corresponding to the numerator wavelengths for predicting calculated protein and Kjeldahl protein, respectively. Phenylalanine has an absorption band at 2177.6 nm but not at 2062.4 nm. Therefore, a measurement at 2177.6 nm records both the phenylalanine and protein, while a measurement at 2062.4 nm the protein only. Thus, the results of NIR technique show a high correlation to Kjeldahl protein at 2177.6 nm and a high correlation to calculated protein at 2062.4 nm. In this study, the NIR technique provides an adequate discrimination for protein in spite of large variations in non-protein nitrogen. This discrimination is, of course, dependent on the availability of a protein absorption band at a wavelength where the non-protein nitrogen gives only a weak band or non at all. Since protein has several strong absorption bands in the near-infrared region, there is a high probability that such a coincidence can be found with other non-protein nitrogen sources.

The optimum numerator wavelength for correlating to phenylalanine, 1140.8 nm, is the wavelength at which phenylalanine has a strong second derivative signal, and both wheat gluten and cellulose have very weak second derivative signals. This explains why a high correlation ($r = 0.995$) is obtained at this wavelength.

The optimum numerator wavelength for correlating to cellulose, 2336.0 nm, is a wavelength at which cellulose has a strong second-derivative signal and both gluten and phenylalanine have very weak second-derivative signals. Since these three components are the only variables in this experiment, a very high correlation ($r = 0.999$) is obtained.

The calibration results of this study have not been tested against unknown samples, but the very high correlation coefficients indicate that it should be possible to predict real protein and Kjeldahl protein independently.

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# ATTEMPTS TO ELABORATE A NON-DESTRUCTIVE OPTICAL METHOD FOR MEASURING THE RIPENESS OF MAGYAR KAJSZI APRICOTS

A. Czabaffy

Central Food Research Institute
H-1022 Budapest, Herman Ottó út 15. Hungary

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The optical characteristics of Magyar kajszi apricots (Armeniaca vulgaris Lam cv. Magyar legjobb = Hungarian best) were studied at the Central Food Research Institute in order to elaborate a non-destructive method for measuring ripeness.

A Neotec 6450 Research Composition Analyser was used for measuring, storing and evaluating the spectrum data of samples obtained from the Research Institute for Fruit Growing and Ornamental Plants (Budapest), hereafter referred to as RIFGOP. A sensory assessment of the ripeness of the samples was made at picking by experts from RIFGOP. The diffuse transmittance spectra of a total of 500 individual fruits were recorded in the course of 1980 and 1981. Regression calculations were carried out to determine the relation between the transmittance values measured and transformed at various wavelengths in the visible range of the spectrum and the ripeness values obtained from sensory evaluations. The calculations were carried out for the data of both years and various types of calibration equations were generated on the basis of the calculations. The correlation coefficients for the calibration equations ranged from 0.84 to 0.93 when referred to individual fruits and from 0.934 to 0.988 when referred to group averages.

One of the calibration equations was selected and used experimentally for grading fruits according to ripeness.

Colour photos were taken of the samples and of the grading in order to conserve to some extent the visual impression they made.

The photograph of the grading experiment shows that the instrumental ripeness prediction carried out using the calibration equation is not bad, and that it is possible to use the diffuse transmittance measured at two wavelengths as a basis for grading apricots according to ripeness or for instrumental ripeness prediction. This is confirmed by statistical calculations, too.

The spectra of apricots at various stages of ripeness indicate that the prediction to ripeness is based on changes caused by the decomposition of chlorophyll in the fruit in the spectrum data in the course of ripening.

**Keywords:** ripeness of apricot, optical ripeness prediction, non-destructive ripeness prediction

Hungary has a long tradition of fruit growing. There is an ever increasing demand for a rapid and, if possible, non-destructive method of determining the quality of fruit; the method should be objective, or at least more objective than the present methods. In order to solve this problem studies on the optical spectra of fruits were initiated at the Central Food Research Institute.

Preliminary experiments carried out in 1979 were reported by Kaffka and Czabaffy (1981). The paper gave a summary of the literature on methods

and equipment used for classifying fruits. Since then, or in addition to this, FLOYD and O'BRIEN (1978) reported on the development of fruit-grading equipment controlled by a microprocessor, and KRIVOSHIEV (1974) gave an account of an automatic tomato grader. KRIVOSHIEV and co-workers (1977) reported on the grading of fruits and vegetables, while KRIVOSHIEV and CHALUKOVA (1981) published data on the characters of the spectra of various fruits, including apricots. CZABAFFY (1984) studied the trasmittance spectra of cherries.

The object of the present work is to elaborate a non-destructive optical method of predicting the ripeness of Magyar kajszi apricots (Armeniaca vulgaris Lam cv. Magyar legjobb = Hungarian best), to determine which calibration equations are suitable for predicting ripeness, to study the statistical characters of these equations and to determine the most important requirements which must be met by the optical measuring head of a one-purpose instrument.

The paper is based on the results of work carried out in previous years. The nature of ripeness has not been investigated; data determined by experts on the basis of sensory assessment were accepted as a measure of ripeness.

## 1. Materials and methods

The samples used in the investigations were supplied by RIFGOP. The samples were grown in the Frank farmstead orchard of the Sasad Horticultural Cooperative at Budaörs and experts from RIFGOP carried out a sensory assessment of their ripeness as they were being picked. The ripeness of groups of samples was determined according to the relevant HUNGARIAN STANDARD (1974) on the basis of skin colour and was expressed as a percentage. In 1980 sixteen sample groups were obtained. The sample groups differed either in degree of ripeness or in date of picking. Each sample group consisted of 20 fruits judged to be of identical ripeness, so a total of 320 fruits were available. In 1981 fifteen groups were obtained, each consisting of 12 fruits, so a total of 180 fruits were available.

The sample groups were arranged on a white plate in order of estimated ripeness and were photographed.

Four to seven hours passed between picking the samples and recording the spectra. The geometrical arrangement used for recording the spectra of the samples is shown in Fig. 1. The apricots were placed on a diaphragm with an aperture of 35 mm in diameter and put one by one into the measuring box of the Neotec 6450 Research Composition Analyser (USA). The diffuse transmittance was measured in the 380–730 nm range. The instrument automatically produced the log $1/T$ values. where $T$ is the ratio of the detected photoelectrical signals caused by light reaching the detector through the diaphragm with

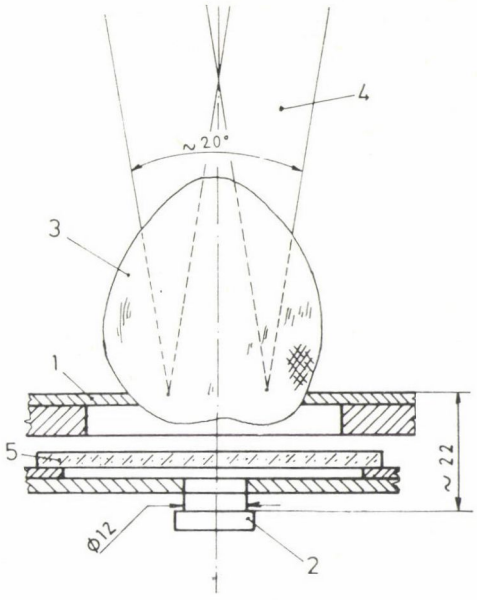


Fig. 1. Geometrical arrangement used for the measurements. 1: diaphragm, 2: Si detector, 3: sample, 4: illuminating beam, 5: glass window

and without fruit placed on the diaphragm. The log $1/T$ spectrum data thus obtained were stored on floppy discs. Figure 2 illustrates the log $1/T$ spectra of apricots at different degrees of ripeness.

A significant change in the spectrum depending on the degree of ripeness was only observed at wavelengths longer than 500 nm, so the calculations described below are confined to this range of wavelengths. On the graph, the lower curve is the spectrum of an over-ripe apricot, and the upper curve is that of an under-ripe apricot, while the intermediate curves represent fruits with intermediate degrees of ripeness. The effect of the decomposition of chlorophyll, having an absorption maximum at around 670 nm, on the shape of the spectrum can be clearly traced in the figure.

The calculations required to process the spectral data were carried out using a NOVA III computer built into the Research Composition Analyser.

While processing the spectral data of samples taken in 1980, the aim was to find two wavelengths where the correlation between the measured spectral data and the ripeness values assessed sensorially and given as percentages, was the greatest. The best correlation was sought in the form of two types of regression equation.

Type $A$ equation is as follows:

$$R = k_0 + k_1 \log \frac{1}{T(\lambda_1)} + k_2 \log \frac{1}{T(\lambda_2)}$$

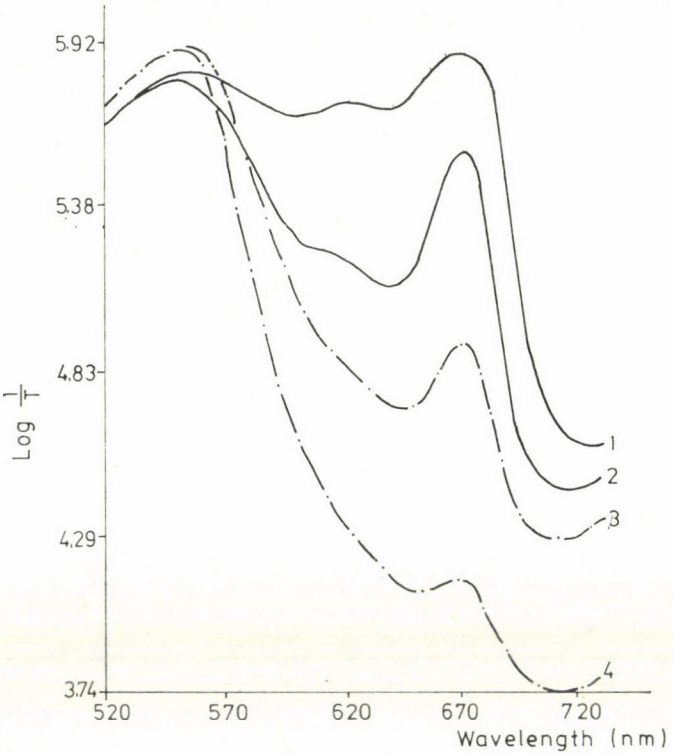


Fig. 2. Typical apricot spectra recorded using a Neotec Research Composition Analyser. 1: unripe (70%), 2: less unripe (80%), 3: almost ripe (94%), 4: slightly over-ripe (104%)

while type $B$ is:

$$R = k_0 + k_1 \frac{\log \frac{1}{T(\lambda_1)}}{\log \frac{1}{T(\lambda_2)}}$$

where $R$ is the ripeness estimated by the equation, $T$ the diffuse transmittance measured at wavelengths $\lambda_1$ and $\lambda_2$, and $k_0$, $k_1$ and $k_2$ the constant and coefficients obtained as the result of the regression calculation.

The procedure used to select the best correlated pair of wavelengths for an equation of type $A$ has been reported previously by CZABAFFY (1983). This method was used to generate the calibration equation labelled MAKH, which applies transmittance data measured at 608 and 643 nm ($r = 0.977$).

When computing the multiple correlation spectrum for the 643 nm wavelength it was observed that, in addition to the maximum found at 608 nm,

another peak of almost the same height is to be found at 578 nm. Using this as one of the pair of wavelengths instead of 608 nm a good, almost identical correlation coefficient was obtained ($r = 0.979$). These wavelengths are used in the calibration equation labelled MAKE.

In generating both the MAKH and the MAKE calibration equations the spectral data of half the individual fruits were applied.

After this the spectral data measured at the same wavelength for all the individuals in each sample group were averaged to give a characteristic mean spectrum for each of the sample groups. When the method of choosing optimum wavelength pairs was applied to these mean spectra the 578 nm and 639 nm wavelength pair was found to be optimum.

When applying the type *B* equation to the spectral data of individual fruits, several wavelength pairs with almost equally high correlations were found, ($r = 0.91 \ldots 0.93$) any of which could be taken as optimum. The calibration equation labelled MAKG was generated using the 640 and 597 nm wavelength pair, while the MAKF equation uses the 690 and 710 nm pair. For the generation of these B type calibration equations the spectra of half the individuals were used.

The spectral data of the other half of the individuals were used to check the calibration equations by substituting these data into each calibration equation and by calculating the statistical characters of each calibration equation using these data, too. This was done partly to confirm that the groups had been halved in a statistically random manner; in addition, the halving of the groups was inevitably necessary, since the available computer could not handle data files consisting of more than 200 spectra at one time. As a control, the data of the mean spectra were also substituted into the calibration equations generated from the spectral data of the individuals, in order to compare the statistical characters calculated using these averaged data to the statistical characters of the MAKC calibration equation, generated on the basis of the means. Naturally, the spectra used in this control belonged to fruits of the same year as those used for generating the calibration equations, so they are not suitable for demonstrating the possible dependence of the calibration equations on the year. This procedure can therefore not be considered as a true control; therefore, the statistical characters thus gained are reported as calibrational data in the results.

For each calibration equation the attainable reproducibility was determined by taking two individuals, each at different stages of ripeness in and out of the instrument twenty times, recording the spectrum on each occasion; the estimated ripeness of the fruit was calculated by substituting the spectral data into the calibration equations, after which the standard deviation of the estimated ripeness data was determined separately for the two individuals as a characteristic of the reproducibility attainable with each calibration equation.

A real check on the calibration equations was carried out in two ways using fruits gathered in 1981. One method was to predict the ripeness of the fruits recording the spectra of individual fruits and substituting the spectral data into the calibration equations; the predicted ripeness data thus obtained were used to calculate the statistical characteristics of the predictions. The second control method was to carry out this procedure using the data of averaged spectra.

Two individuals at different stages of ripeness were again selected from the second year's harvest and the reproducibility of each calibration equation was determined in the manner described above.

The MAKE calibration equation was also used to classify the ripeness of sensorially unassessed individuals from the 1981 season. A colour photo was taken of the result.

In the MAKE calibration equation: $k_1 \simeq k_2$. This suggested that a simpler calibration equation, namely

$$R = k_0 + k_1 \left( \log \frac{1}{T(\lambda_1)} - \log \frac{1}{T(\lambda_2)} \right)$$

could be generated with good results. Hereafter, this type of calibration equation will be called type *D*. The software development carried out at the Central Food Research Institute made it possible to carry out regression calculations with equations of this type, too. The regression calculations were carried out for both the individual spectrum data and the averaged spectrum data. On the basis of the data obtained from the calculations, and drawing on earlier experience, six different types of *D* calibration equations were generated. The three pairs of wavelengths used in the calibration equations were selected in the following manner.

Type *D* regression calculations were carried out on the spectrum data of individuals from the 1980 season using the wavelength pairs belonging to the two type *A* calibrations, namely MAKE and MAKH, which best fitted the individuals from the 1980 season. The correlation coefficients were then calculated. Investigations were then made to discover whether these correlation coefficients differed substantially from those calculated using the wavelength pair which proved to be mathematically optimum. The optimum wavelength pair was found to be 642 and 598 nm with a correlation coefficient of 0.930, while the MAKE wavelengths gave a value of 0.926 and the MAKH wavelengths a value of 0.928. Since these do not differ from the optimum, the previously selected wavelength pairs were judged to be suitable for the setting up of type *D* calibration equations, too. The wavelength pair found in equation MAKE was thus used to generate the calibration equations MAKE2 and MAKEE, the former using individual spectrum data and the

latter using averaged spectrum data, while the wavelength pair from calibration equation MAKH was used to generate calibration equations MAKH2 and MAKHH. In the case of averaged spectrum data the correlation coefficient for the mathematically optimum wavelength pair was 0.982, while that of the actual MAKEE calibration equation was 0.977 and that of the MAKHH equation 0.974.

The calibration equation BARA2 was formed using the wavelength pair giving the maximum correlation for the spectrum data of individuals from the 1981 season, and the BARAA equation with the same wavelength pair but using the averaged spectrum data.

In order to check these type *D* calibration equations, the averaged spectrum data of the season not used for generating the equations and the spectrum data of the individuals of both seasons were separately substituted into the calibration equations generated on the basis of averaged spectrum data, while the individual spectrum data of the season not used to generate the equations and the averaged spectrum data of both seasons were separately substituted into the calibration equations generated on the basis of individual spectrum data; the statistical characteristics of the predictions thus gained were then calculated.

## 2. Results

Table 1 gives a summary of the sensorially assessed ripeness data for the 1981 sample groups, the ripeness data predicted for these groups on the basis of the calibration equations generated using spectrum data from the 1980 samples, the standard deviation of predicted ripeness within the sample groups using the same calibration equations, and the reproducibility achieved with each calibration equation.

Values greater than 100% can be found in the columns giving predicted ripeness data. These values are interpreted as indicating that the sample or group of samples in question is riper than what was judged by the experts to be of optimum ripeness for immediate consumption.

It can be seen from the table that the MAKF equation, which uses transmittance values measured at wavelengths close to the absorption peak of chlorophyll and which could thus be expected to indicate the ripening process well, behaves differently from the other calibration equations. This difference in behaviour is exhibited as an extremely high standard deviation in the least ripe group and as an extremely low standard deviation in the ripe groups. This can probably be attributed to the great difficulty encountered in taking measurements in the neighbourhood of the chlorophyll absorption peak due to the fact that the absorption changes rapidly with the wavelength.

Table 1

*Ripeness data obtained for 1981 sample groups of the variety Magyar legjobb (Hungarian best) using sensory assessment and various calibration equations*

| Sample groups | Sensorially assessed ripeness of sample groups as a percentage | Instrumentally predicted ripeness of sample groups as a percentage using calibration equation labelled | | | | | Instrumentally predicted standard deviation as a percentage of ripeness within groups using calibration equation labelled | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MAKC | MAKE | MAKF | MAKG | MAKH | MAKC | MAKE | MAKF | MAKG | MAKH |
| BAR20 | 65 | 65 | 69 | 70 | 67 | 68 | 3.1 | 2.7 | 6.0 | 2.3 | 2.7 |
| BAR10 | 70 | 74 | 76 | 79 | 74 | 75 | 5.6 | 4.5 | 3.9 | 3.7 | 3.9 |
| BAR30 | 65 | 73 | 76 | 82 | 75 | 76 | 7.5 | 6.1 | 5.9 | 5.6 | 5.6 |
| BAR21 | 75 | 78 | 79 | 80 | 78 | 78 | 6.8 | 5.9 | 7.3 | 5.2 | 5.8 |
| BAR31 | 75 | 82 | 83 | 87 | 82 | 82 | 5.4 | 4.2 | 3.8 | 3.7 | 3.4 |
| BAR12 | 80 | 82 | 84 | 85 | 83 | 84 | 6.0 | 5.5 | 5.2 | 5.2 | 5.5 |
| BAR22 | 85 | 87 | 87 | 89 | 86 | 87 | 5.9 | 5.1 | 3.8 | 3.9 | 3.8 |
| BAR11 | 90 | 90 | 91 | 93 | 90 | 91 | 8.5 | 6.8 | 4.1 | 5.5 | 5.3 |
| BAR32 | 85 | 93 | 93 | 93 | 92 | 92 | 6.4 | 4.2 | 2.1 | 3.3 | 3.3 |
| BAR23 | 95 | 96 | 95 | 94 | 95 | 95 | 6.5 | 4.9 | 2.9 | 5.0 | 4.8 |
| BAR33 | 95 | 95 | 95 | 96 | 94 | 94 | 4.7 | 3.5 | 2.2 | 2.8 | 2.4 |
| BAR13 | 95 | 93 | 95 | 98 | 96 | 95 | 9.9 | 6.8 | 1.4 | 5.7 | 5.0 |
| BAR34 | 100 | 94 | 97 | 99 | 99 | 97 | 5.5 | 3.5 | 0.8 | 3.3 | 3.0 |
| BAR24 | 100 | 100 | 100 | 99 | 100 | 99 | 8.0 | 5.4 | 1.1 | 4.9 | 5.2 |
| BAR14 | 100 | 99 | 101 | 99 | 102 | 100 | 8.1 | 5.1 | 1.4 | 4.2 | 3.6 |
| | | Predicted ripeness of fruits used for testing the reproducibility | | | | | Reproducibility | | | | |
| BARX | | 69 | 70 | 63 | 68 | 68 | 0.8 | 0.7 | 2.2 | 0.6 | 1.0 |
| BARY | | 101 | 99 | 99 | 99 | 99 | 2.3 | 1.6 | 0.3 | 0.8 | 0.7 |

The standard deviation of the slightly different prediction results obtained in the course of repeated measurements on individuals BARX and BARY is given to show the reproducibility.

The ripeness predicted by calibration equation MAKC has a considerably higher within-group standard deviation than that predicted using the other equations. This could be due to the fact that this calibration equation was generated using averaged spectrum data.

Calibration equations MAKE, MAKG and MAKH gave practically identical predictions of the ripeness of the sample groups; the difference between them did not exceed 2%.

In Tables 2 and 3 the reference year of calibration equations marks the year of fruit growing, the spectra of which were used to generate the given calibration equations.

Table 2 contains the statistical characteristics of calibration equations generated on the basis of 1980 samples. Not only the characteristics calculated when the equations were generated, but also those calculated when the equations were applied to predict the ripeness of 1981 fruits are listed. In addition

Table 2

*Statistical data of type* A *and* B *calibration equations generated by the 1980 samples and those obtained when checking these equations using 1981 samples*

| Label of calibration equation | | MAKC | MAKE | MAKF | MAKG | MAKH |
|---|---|---|---|---|---|---|
| Type of equation | | A | A | B | B | A |
| Wavelengths (nm) | | 578; 639 | 578; 643 | 690; 710 | 640; 597 | 608; 643 |
| Equation based on | | group averages | individuals | individuals | individuals | individuals |
| At calibration for fruit group averages | SEC | 3.0 | (3.4) | (3.9) | (3.5) | (3.4) |
| | *r* | 0.981 | (0.979) | (0.968) | (0.974) | (0.977) |
| At calibration for individual fruits | SEC | (5.7; 6.0) | 5.2; (5.4) | 5.7; (5.7) | 5.1; (5.2) | 4.9; (5.1) |
| | *r* | (0.917) (0.913) | 0.926 (0.922) | 0.912 (0.910) | 0.928 (0.926) | 0.934 (0.930) |
| | bias | (0.4; 0.2) | (0.5) | (0.4) | (0.5) | (0.5) |
| reproducibility | | 0.8; 0.4 | 0.6; 0.4 | 0.8; 0.1 | 0.6; 0.3 | 0.9; 0.5 |
| At prediction for fruit group averages | SEP | 4.1 | 4.3 | 5.4 | 3.4 | 4.0 |
| | *r* | 0.964 | 0.970 | 0.942 | 0.976 | 0.974 |
| At prediction for individual fruits | SEP | 6.9 | 5.9 | 6.2 | 5.1 | 5.2 |
| | *r* | 0.836 | 0.879 | 0.868 | 0.909 | 0.908 |
| | bias | 1.8 | 3.0 | 4.7 | 2.5 | 2.6 |
| reproducibility | | 0.8; 2.3 | 0.7; 1.6 | 2.2; 0.3 | 0.6; 0.8 | 1.0; 0.7 |

SEC: standard error of calibration; SEP: standard error of prediction; *r*: correlation coefficient. The values of SEC, SEP and bias, and the reproducibility, are given as ripeness percentages. The calibration equations generated on the basis of group averages were tested to predict the ripeness of individuals and the equations generated on the basis of individuals were tested to predict the ripeness of group averages; data gained in this way are given in brackets as calibration data. For explanation of the labels of the calibration equations see "Materials and methods".

to the statistical characteristics obtained when the equations were generated, statistical characteristics obtained by substituting individual or averaged spectrum data which were not used to generate the equations but which originate from fruit of the reference year are given in brackets. On studying the data obtained at prediction it can be seen that the MAKF calibration equation gives the worst prediction of group averages and also gives the greatest bias. For individuals the MAKC calibration equation gives the worst prediction ($r = 0.836$). The other three equation sgive practically identical predictions, with the MAKG calibration equation giving formally the best of all ($r = 0.909$).

Table 3 summarises the calibration equations generated as the result of type *D* regression calculations.

Table 3

*Data of type* D *calibration equations generated for apricot samples of the variety Magyar legjobb (Hungarian best)*

| Label of calibration equation | | MAKE2 | MAKEE | MAKH2 | MAKHH | BARA2 | BARAA |
|---|---|---|---|---|---|---|---|
| Type of equation | | D | D | D | D | D | D |
| Wavelengths (m) | | 578; 643 | 578; 643 | 608; 643 | 608; 643 | 636; 650 | 630; 650 |
| Equation based on | | individuals | group averages | individuals | group averages | individuals | group averages |
| Reference year | | 1980 | 1980 | 1980 | 1980 | 1981 | 1981 |
| Constant $k_0$ of calibration | | 67.2 | 65.4 | 69.2 | 67.8 | 80.2 | 79.5 |
| Coefficient $k_1$ equations | | 30.2 | 33.0 | 82.6 | 90.0 | 150.0 | 169.6 |
| At calibration | | | | | | | |
| for fruit group averages | SEC | (3.3) | 3.0 | (3.4) | 3.2 | (3.4) | 2.1 |
| | $r$ | (0.979) | 0.977 | (0.977) | 0.974 | (0.988) | 0.987 |
| | bias | (0.5) | — | (0.4) | — | (1.4) | — |
| for individual fruits | SEC | 5.2 | (5.4) | 5.1 | (5.3) | 4.6 | (4.6) |
| | $r$ | 0.926 | (0.926) | 0.929 | (0.930) | 0.928 | (0.929) |
| | bias | — | (0.0) | — | ( 0.1) | — | (1.6) |
| reproducibility | | 0.6; 0.4 | 0.7; 0.4 | 1.0; 0.8 | 1.1; 0.9 | 2.2; 0.9 | 2.5; 1.9 |
| At prediction | | | | | | | |
| for fruit group averages | SEP | 4.2 | 3.7 | 3.9 | 3.4 | 7.2 | 6.4 |
| | $r$ | 0.970 | 0.970 | 0.973 | 0.973 | 0.934 | 0.934 |
| | bias | 3.3 | 3.6 | 1.8 | 1.9 | 3.9 | 4.1 |
| for individual fruits | SEP | 5.9 | 5.9 | 5.5 | 5.6 | 7.8 | 7.6 |
| | $r$ | 0.879 | 0.879 | 0.894 | 0.894 | 0.849 | 0.849 |
| | bias | 3.3 | 3.6 | 5.5 | 1.9 | 3.7 | 3.8 |
| reproducibility | | 0.7; 1.6 | 0.6; 1.7 | 1.1; 0.9 | 1.2; 1.0 | 1.4; 1.5 | 1.6; 1.7 |

For symbols and other explanations, see Table 2

It can be seen from the table that when the ripeness of fruits from the 1980 season is predicted using the calibration equations BARA2 and BARAA, which contain the wavelength pair found to be optimum for the 1981 season, the correlation coefficients decrease substantially compared to the values obtained at calibration; namely, from around 0.98 to around 0.93 for the group averages, and from around 0.93 to 0.84 for individuals. Accordingly, the SEP values considerably exceed the SEC values. When the calibration equations using the 1980 data are used to predict the ripeness of fruits from the 1981 season, the reduction in the correlation coefficients and the increase in SEP are considerably smaller. It is quite clear from the table that the calibration equations generated using wavelength pairs which proved to have mathe-

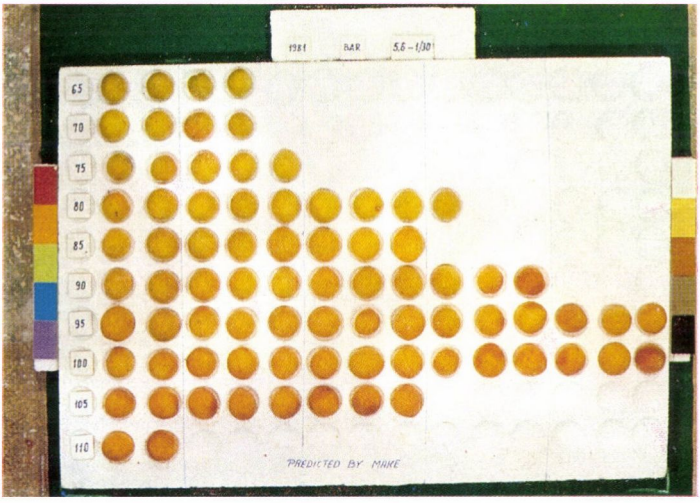


Fig. 3. Photograph taken of apricots graded according to ripeness. The column of figures on the left indicates the nominal ripeness of the rows. Sorting was carried out using calibration equation MAKE. The figures at the top indicate the year, number, identity mark, and the aperture and exposure time used when taking the photograph

matically maximum correlation for the 1981 spectrum data fit the ripeness of 1980 samples far less well than the calibration equations generated with wavelength pairs selected by the 1980 samples and lying far from the mathematical optimum of the 1981 samples fit the ripeness of the 1981 samples.

The table also includes data characterizing the reproducibility determined using the calibration equations. These data indicate that the reproducibility of calibration equations generated on the basis of the 1981 samples is also worse (1.55%) than that of those generated on the basis of 1980 samples (1.11%).

Type *A* regression calculations were also carried out for the 1981 samples using averaged spectrum data. The wavelengths giving maximum correlation with ripeness were 588 and 663 nm. However, the statistical characteristics obtained using these wavelengths were not better than those of the type *D* calibration equation BARAA, so these are not included in the table. (Data for wavelengths 588 and 663 nm: SEC = 2.2; $r = 0.987$.)

Comparing the statistical characteristics obtained at prediction for the MAKE calibration equation in Table 2 and the MAKE2 and MAKEE equations in Table 3. it can be observed that they are practically equivalent. Apart from the bias, the MAKH calibration equation is also equivalent to MAKH2 and MAKHH.

The photograph taken of grading according to ripeness is presented in Fig. 3. The apricots were selected on the basis of the MAKE calibration equation. In the figure, fruits predicted instrumentally to be of approximately the same ripeness are placed in horizontal rows. The figure on the left of the

row indicates the nominal ripeness of the row. The fruits placed in each row had an instrumentally predicted ripeness deviating only by $\pm 2.5\%$ from the nominal value.

## 3. Conclusions

It can be seen from the data in Table 3 that calibration equations generated on the basis of spectrum data of individuals and those generated on the basis of spectrum data averaged within a ripeness group for a given season predict the ripeness of individuals and group averages from the other season with practically identical statistical characteristics, i.e. calibration equations MAKE2, MAKH2 and BARA2 are equivalent to equations MAKEE, MAKHH and BARAA, respectively.

In the calibration equations generated from averaged spectrum data, coefficient $k_1$ is always approx. 10% greater than in calibration equations generated from spectrum data of individuals using the same wavelength pairs. The constant $k_0$ behaves in reverse, being smaller in equations generated from averaged data. In spite of this, however, in the range of ripeness in question when substituting identical spectrum data into calibration equations generated in these two different ways there is less than 3% difference in the predicted ripeness values.

The fact that calibration equations generated using the 1980 data give a better prediction of the ripeness of 1981 fruits than calibration equations generated by 1981 data do for the ripeness of 1980 fruits indicates the important fact that wavelengths selected mathematically by an objective method and giving maximum correlations, also carry subjective, random information, since the wavelength pair found to be optimum is dependent not only on objective spectrum data, but also on the subjective assessment of the experts, on the ripeness range covered by the fruit available, and on the ripeness of the sample groups placed more or less randomly within this range. It follows that, as long as the assessment of ripeness remains subjective, this subjectivity will be carried by the calibration equations which appears in objective form.

It should be noted, however, that part of the deviation between the sensory assessment made on the basis of skin colour and the instrumental prediction carried out on the basis of the transmittance spectrum of the flesh is definitely objective due to the methodological differences.

Nevertheless, the grading experiment indicates that even the mathematically simpler type $D$ calibration equations satisfy present requirements, though it is desirable to test them for grading fruits from further years.

The wavelengths to be found in the calibration equations MAKE2 or MAKH2 can be recommended for use in constructing a one-purpose instrument. The wavelengths found in calibration equation MAKG, which was

formally the best of all, can also be recommended, but since this is a type *B* equation the construction of the equipment will be more complicated. The transmittance values to be measured can be seen in Fig. 2. These cover several orders of magnitude and the measurements make heavy demands on the optical measuring head if the instrument is to be used industrially, since the measuring head must be protected from environmental scattered light, or the effect of such light must be eliminated.

The attempts made so far to determine the effect of fruit size on ripeness prediction and to express this numerically, so that the size dependence could be incorporated into the calibration equations, have been in vain. The only acceptable explanation for this seems to be that the uncertainty of sensory assessments of ripeness exerts a greater effect on the results of correlation calculations than changes in spectrum data caused by differences in the size of the fruit. This means that the uncertainty of the sensory assessments makes it impossible to demonstrate spectrum data changes resulting from differences in fruit size by simple means.

This problem, together with the effect on the spectrum data of the temperature and growing site of the samples and the time elapsed since picking, will require further study only if a demand arises for more accurate predictions.

Importance has been attached throughout this work to determining the correlation between the quality characters (e.g. dry matter content, carbohydrate content, etc.) of the fruit and the sensorially assessed ripeness, and between the quality characters and the ripeness predicted by the non-destructive method elaborated. These studies are already in progress and the results will be reported in a later paper.

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# IN VITRO STUDIES ON THE EFFECT OF THE COMBINATION TREATMENT OF HEAT AND IRRADIATION ON SPORES OF *ASPERGILLUS FLAVUS* LINK NRRL 5906

G. T. ODAMTTEN[a,c], V. APPIAH[a,d] and D. IS. LANGERAK[b,e]

[a] International Facility for Food Irradiation Technology c/o Pilot Plant for Food Irradiation, P.O.Box 87, 6700AB Wageningen. The Netherlands
[b] Research Institute ITAL, P.O.Box 48, 6700AA Wageningen. The Netherlands.
[d] Department of Biology, Food and Agriculture, National Nuclear Research Centre, Ghana Atomic Energy Commission, P.O. Box 80 Legon/Accra. Ghana
[e] State Institute for Quality Control in Agricultural Products, P.O. Box 230, 6700 AE Wageningen. The Netherlands

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Studies have been carried out to investigate the effect of the combination treatment of heat and subsequent gamma irradiation on the survival of conidia of *A. flavus* Link NRRL 5906. The mould spores were heat-treated either in aqueous suspension (containing 0.2% Tween-80) or harvested dry and heat-treated by hot air in a climate chamber at low humidity (≤45% R.H.) or high humidity (≥85% R.H.). Heat treatments of the aqueous suspensions were performed at 20, 40, 45, 48, 50, 52, 53, 55, 58, 59 and 60 °C for 2.5, 5.0 and 10 minutes, respectively. Hot-air treatment of dry-harvested spores was done for 30 minutes at 60 °C. Suspensions were given 0.25, 0.50, 0.70, 1.0 and 1.5 kGy of gamma radiation, while dry harvested spores were exposed to 3.5 and 4.0 kGy. Irradiation was carried out not later than 30 min after heat treatment.

In aqueous suspension the adverse effect of heating on the spores generally started after being at the temperature of 50 °C or higher for at least 2.5 min. Heating for at least 5 min at 59 °C inactivated the moistened spores completely (at least 5 log cycles reduction in the viable count on maize meal agar). When heating of the suspensions was combined with a subsequent irradiation treatment, a synergistic effect was observed at a temperature above 50 °C and the synergism increased with increasing heat damage (increasing the heating time and/or temperature).

Heating of dry conidia at 60 °C for 30 min at high humidity (≥85% R.H.) reduced their viable count by 3 log cycles, whilst a parallel treatment at low humidity (≤45% R.H.) did not appreciably reduce the viable mould spores. However, when the spores were stored at 80% R.H. for 4 days before plating, a considerable fraction recovered from the heat damage. Radiation treatment was more effective after exposing the spores to hot air with high humidity (≥85% R.H.) before irradiation than when low humidity (≤45% R.H.) was applied. The practical implications of these findings are discussed.

**Keywords:** Combined treatment, irradiation of spores, survival of *A. flavus*

The contamination of food consumed by humans and animals alike by fungi has raised much concern, particularly those fungi which produce potent mycotoxins (ENOMOTO & SAITO, 1972; AUSTWICK, 1975; TUINSTRA et al., 1975; SMITH et al., 1976; HARWIG et al., 1979; HUFF et al., 1979; ZUBER

[c] Present address: Department of Botany, University of Ghana, P.O.Box 55, Legon/Accra. Ghana

& LILLEHOJ, 1979; ABALAKA & ELEGBEDE, 1982). Prominent amongst these is *Aspergillus flavus* which produces the noxious and carcinogenic metabolites known as aflatoxins.

The use of gamma radiation alone for disinfestation of cereals and control of fungi infecting cereals and grains is well documented (KULIK & JUSTICE, 1966; POISSON & CAHAGNIER, 1974; KISS & FARKAS, 1977; ODAMTTEN, 1979, 1982) but it is desirable to undertake in vitro studies pertaining to the radiation response of the spores of *A. flavus* in combination with previous heating.

There are references in the pertinent literature on the novel approach for controlling fungal infection of stored products by the combination treatment of heat and irradiation. Radiation treatment combined with heat treatment (KISS & CLARKE, 1969; ROY et al., 1972; SOMMER et al., 1972; BRODRICK et al., 1976, 1977; LANGERAK & CÃNET-PRADES, 1979) was more effective than radiation treatment alone.

Recently, PADWAL-DESAI and co-workers (1976), showed that different strains of *A. flavus* (toxigenic and non-toxigenic) responded differently to heat treatment alone and to the combined treatment with gamma irradiation. This phenomenon had previously been reported by SOMMER and co-workers (1964). It is likely that the strain *A. flavus* NRRL 5906 infecting maize grains may show different response to heat treatment alone and to combined treatment with gamma irradiation. Such in vitro studies on this fungus using a model medium, maize meal agar (MMA), closely related to the product will provide the most relevant information necessary for the subsequent in vivo application studies on the product, maize grains. These experiments reported here were designed to provide pertinent information on the effect of mild heat (20–60 °C) alone and the combination treatment with gamma irradiation on the survival of spores of *A. flavus* NRRL 5906.

## 1. Materials and methods

### *1.1. Treatment of spores*

*1.1.1. Treatment in suspension* (A). Spores of *A. flavus* Link NRRL 5906 were used in these studies. The method employed was essentially a modification of the procedure of LANGERAK and CANET-PRADES (1979). Spores of *A. flavus* were suspended in sterile Tween solution (0.2% Tween 80). The suspension was centrifuged at 4000 g for 15 min, the supernatant discarded with subsequent resuspending of the pelleted spores in fresh Tween. The centrifugation was repeated. The resultant suspension was then adjusted to a spore density of $10^6$ to $10^7$ spores per $cm^3$ with the help of a Hawksley B. S. 784 Haema cytometer (Hawksley and Sons Ltd., Sussex, England). Heating tubes

(⌀ 2.5 cm, 16 cm long) containing 27 $cm^3$ of sterile Tween solution used for each heating temperature (20, 40, 45, 48, 50, 52, 53, 55 and 60 °C) were maintained at the respective stated temperatures in water bath (P.M. Tamson N. V., Holland) and 3 $cm^3$ aliquots of the stock suspension of spores were added. After heating for the pre-determined time intervals of 2.5, 5.0 and 10 min, the tubes were transferred to room temperature (cca 20 °C). Tubes which contained unheated spores (20 °C) were regarded as controls.

*1.1.2. Treatment at different R. H.* (B). The spores were harvested dry from a maize medium (200 g blended maize in 500 $cm^3$ Erlenmeyer flask) and 0.1 g weight of spores transferred into sterile petri dishes (9.0 cm diameter). The spores were heated at 60 °C for 30 min in a forced heat micro-climatic chamber that enabled us to give the spores either a low humidity ($\leq$45% R. H.) or high humidity ($\geq$85% R. H.) during the heating period (ODAMTTEN et al., 1980). The lids of the petri dishes were eccentrically placed on the bottoms to allow maximum exposure of the spores to the prescribed treatment. The humidity and temperature during the heating period was monitored from a remote position (ODAMTTEN et al., 1980). After irradiation in the dry state, the spores were suspended in 30 $cm^3$ of sterile Tween solution before plating on the agar medium. Other combined treated spores in petri dishes with nipples were kept at 80% R. H. for 4 and 8 days, respectively, before plating on Maize Meal Agar (MMA, 200 g maize powder in 1 $dm^3$ distilled water) and Oxytetracycline Glucose Yeast Extract Agar Medium (OGYE, oxytetracycline 0.5 g; glucose 20 g; yeast extract 5 g; distilled water 1 $dm^3$).

### *1.2. Radiation treatment*

The irradiation was carried out not more than 30 min after heat treatment in the Pilot Plant for Food Irradiation at the International Facility for Food Irradiation Technology (IFFIT) Wageningen, The Netherlands. The dose rate was 2.6 kGy $h^{-1}$. The absorbed dose was checked by Fricke's dosimetry and clear perspex dosimeters. Test tubes containing 4.0 $cm^3$ aliquots of heat-treated moist spores (Treatment A) were given 0.20, 0.50, 0.70 and 1.0, 1.5 kGy of gamma irradiation, resp., whilst the dry spores (Treatment B) were exposed to 3.5 and 4.0 kGy of gamma radiation.

### *1.3. Assessment of survivors in treated spores*

Aliquots of 1.0 $cm^3$ of the combination treated spores were diluted at the ratio 1 : 9 and were plated out on MMA and OGYE media employing the conventional microbiological technique of serial dilution. After an incubation period of 3 days at 28 °C, the log survival was plotted on linear graph paper against radiation dose. The $D_{10}$ values and correlation coefficients of the combination treated samples were calculated where appropriate.

## 2. Results

### *2.1. Effect of heating and in combination with radiation on spores in Treatment A*

The effects of the various temperatures and heating times in Treatment A are illustrated in Fig. 1. There was no difference in the surviving population in the range of 20–45 °C after 2.5, 5.0 and 10 min heating periods. Subsequent increase in heating temperature from 45 °C to 49 °C for 2.5 and 5.0 min did not reduce the surviving population of spores. The adverse effect of heating on the spores generally started after the spores had been treated at about 52 °C for at least 2.5 min. At temperatures higher than 50 °C, a reduction in the surviving population was recorded when the heating time was increased from 2.5 to 10 min. The spores were, however, completely killed after heating them for 5 min and 10 min at 59 °C and 58 °C, respectively. Thus heating alone

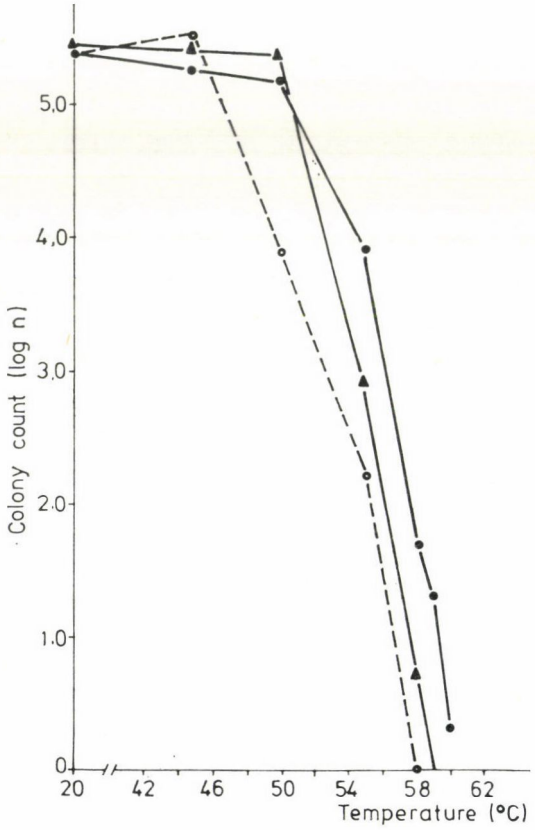


Fig. 1. Graph showing effect of heating temperature and duration of heating on the survival of spores of *A. flavus* in suspension. (Medium used was MMA). —●—: heating for 2.5 min; —▲—: heating for 5 min; ——○——: heating for 10 min

for at least 5 min at 59 °C is considered sufficient to inactivate the spores of *A. flavus* treated in suspension.

When irradiation was (0.25–1.5 kGy) combined with heating, a synergistic effect was obtained even at the sub-lethal temperatures from 50 °C to 53 °C where heating alone had a slight effect on the spores (Figs. 2–4). Generally 5 min heating at 53 °C in combination with 0.75 kGy of gamma radiation resulted in complete inhibition of spore germination (Fig. 2).

The $D_{10}$ values obtained are presented in Table 1. The correlation coefficients were in the range of 0.930 to 0.999.

There was, generally, a decrease in $D_{10}$ values when the sub-lethal heating temperature of 50 °C was exceeded and similar $D_{10}$ values of 0.19 and 0.18 kGy were obtained after combination treatment with 0.75 kGy for 5 and 10 min heating times, resp., at 53 °C.

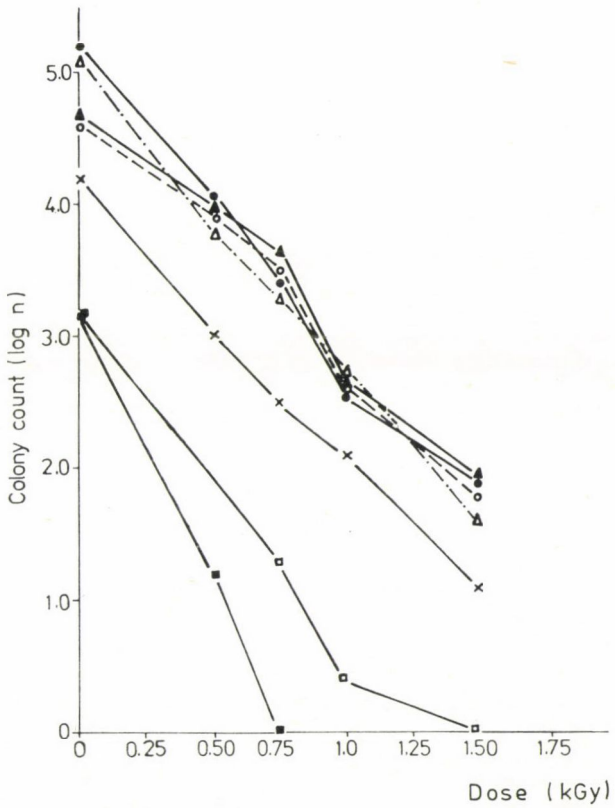


Fig. 2. Effect of 2.5 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.50–1.50 kGy) on the survival of spores. (Incubation medium was MMA.) –●–: 20 °C; ––○––: 40 °C; –▲–: 45 °C; –·–△–·–: 48 °C; –×–: 50 °C; –□–: 52 °C; –■–: 55 °C

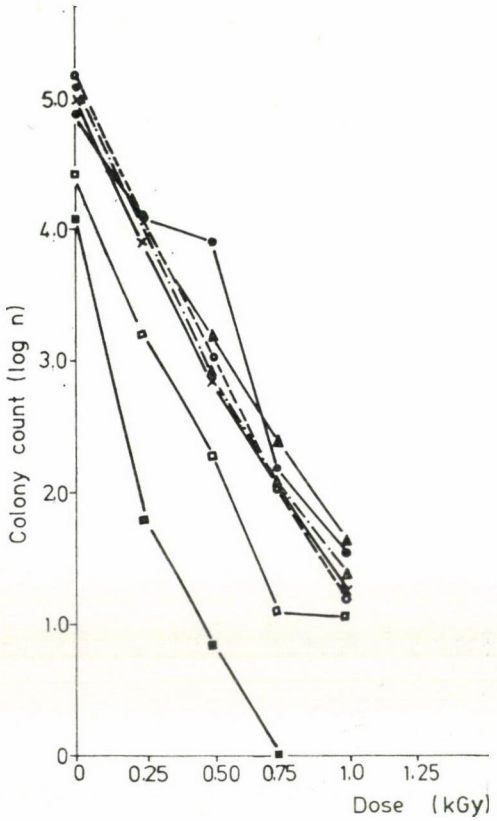


Fig. 3. Effect of 5 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.25–1.0 kGy) on the survival of spores. (Incubation medium was MMA.) -·-●-·-: 20 °C; --○--: 40 °C; —▲—: 45 °C; —△—: 48 °C; —×—: 50 °C; —□—: 52 °C; —■—: 53 °C

Table 1

*Comparative* $D_{10}$ *values (kGy) of combination treatment of heating (different temperatures and times) and gamma radiation, resp.* (Spores of *Aspergillus flavus*)

| Heating temperature (°C) | $D_{10}$ values at indicated heating time periods (kGy) | | |
|---|---|---|---|
| | 2.5 min[a] | 5.0 min | 10 min |
| 20 | 0.43 | 0.29 | 0.25 |
| 40 | 0.52 | 0.28 | 0.28 |
| 45 | 0.51 | 0.27 | 0.29 |
| 48 | 0.44 | 0.27 | 0.25 |
| 50 | 0.50 | 0.29 | 0.25 |
| 52 | 0.42 | 0.29 | 0.19 |
| 53 | — | 0.19 | 0.18 |
| 55 | 0.32 | — | — |

[a] Data for 2.5 min were obtained from a separate experiment

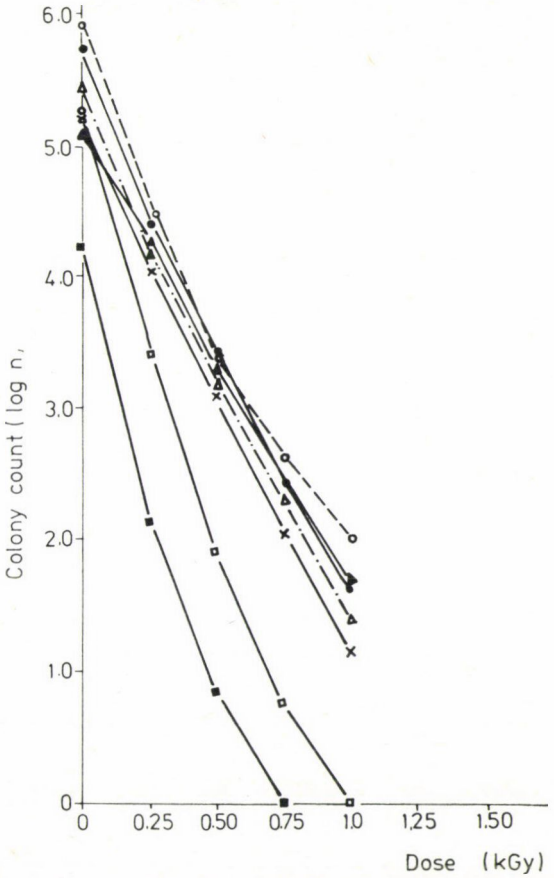


Fig. 4. Effect of 10 min heating of spores of *A. flavus* in suspension at the indicated temperatures, in combination with gamma radiation doses (0.25–1.0 kGy) on the survival of spores. (Incubation medium was MMA.) —●—: 20 °C; --○--: 40 °C; —▲—: 45 °C; —△—: 48 °C; —×—: 50 °C; —□—: 52 °C; —■—: 53 °C

### *2.2. Effect of heating only and in combination with radiation on dry spores in Treatment B*

The effect of heating alone (60 °C for 30 min) and the combination treatment with radiation (0, 3.5 and 4.0 kGy) on the dry spores is illustrated in Figs. 5 and 6. The efficacy of heat in inactivating spore germination depended strongly on the ambient humidity during heat treatment. Heating alone at 60 °C for 30 min at high humidity ($\geq$85% R. H.) reduced the mould count on OGYE (Fig. 5) and MMA (Fig. 6) by 3 log cycles whilst a parallel treatment at low humidity ($\leq$45% R. H.) conditions did not appreciably reduce the

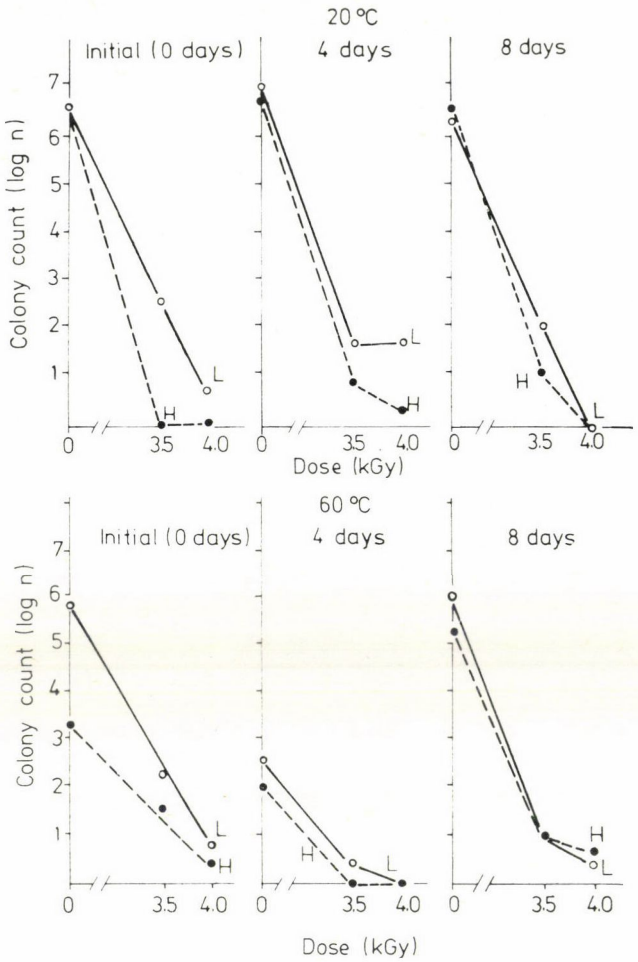


Fig. 5. Effect of irradiation of the survival of unheated (control) spores of *A. flavus* (top) kept after treatment at 80% R.H. ambient humidity for 0, 4, and 8 days, respectively, before plating on OGYE medium; and (bottom) the effect of the combination of heat (60 °C for 30 min) with irradiation on the survival of spores of *A. flavus* stored at 80% R.H. for 0, 4 and 8 days, respectively, before plating on OGYE. L: humidity $\leq$45% R.H.; H: humidity $\geq$85% R.H.

spore population. However, when the combination treated spores were stored at 80% R. H. for 4 days before plating, spore germination was considerably reduced (by 3 log cycles) even under low-humidity heating conditions. The lethality of the combined treatment was evident after 4 days but thereafter, the spores rather curiously recovered after 8 days storage at 80% R. H. Again, similar results were obtained on the two solid media, namely OGYE and MMA.

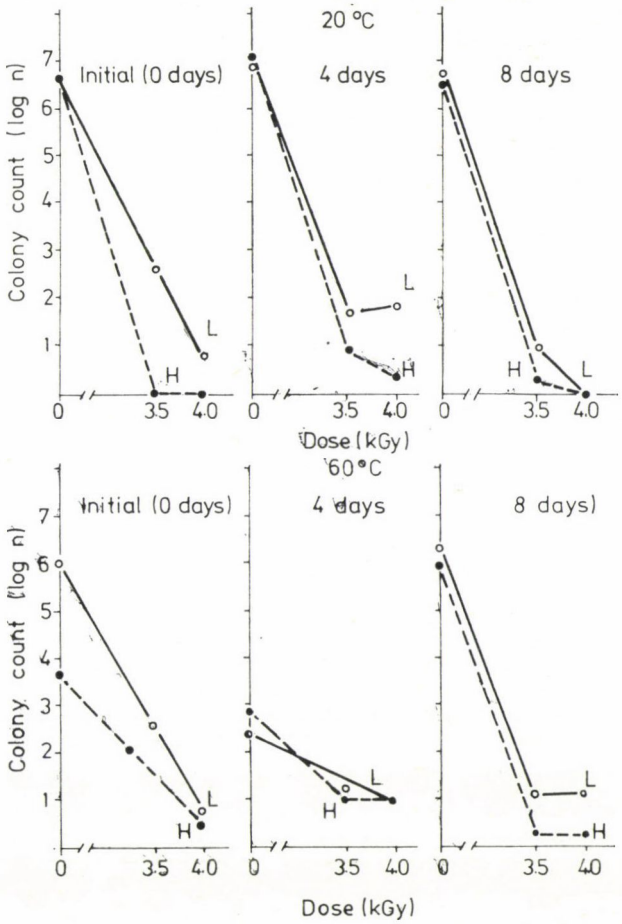


Fig. 6. Effect of irradiation on the survival of unheated (control) spores of *A. flavus* (top) kept, after treatment, at 80% R.H. ambient humidity for 0, 4 and 8 days, respectively, before plating on MMA medium; and (bottom) the effect of the combination of heat (60 °C for 30 min) with irradiation on the survival of spores of *A. flavus* stored at 80% R.H. for 0, 4 and 8 days respectively before plating on MMA medium. L: humidity $\leq$45% R.H.; H: humidity $\geq$85% R.H.

The unheated (20 °C) spore population remained the same with a slight increase on the 4th day at 80% R. H. There was no distinct difference between unheated and unirradiated spores treated under low and high humidity conditions, respectively. In all instances, the size of colonies was larger on OGYE than on MMA.

Radiation treatment was more effective after exposing the spores to hot air of high humidity ($\geq$85% R. H.) before irradiation than when low humidity ($\leq$45% R. H.) was applied (Figs. 5 and 6).

## 3. Conclusions

The report of MOYHUDDIN and SKOROPAD (1975) stated that the synergistic effect of heat and gamma radiation on the conidia of *A. flavus* Link var. *columnaris* Rapper and Fennel increased with an increase in temperature (35–55 °C applied for 20 min) and radiation dose (0.25–1.25 kGy) and that this increase was more pronounced at temperatures above 45 °C. They found that heating the conidia at 50 °C for 20 min followed by irradiation was most effective. At 1.0 and 1.25 kGy gamma radiation, there were no survivors. The conidia of *A. flavus* Link (toxigenic) were more resistant to heat treatment at 50 °C for 5 min than conidia of *A. flavus*, *A. oryzae* (non toxigenic). However, survival of both strains was not affected by being exposed to 50 °C for 75 min (PADWAL-DESAI et al., 1976). Heat treatment at 55 °C for 5 min reduced the conidial population to 6.5% and 0.12% in the toxigenic and non-toxigenic strains, respectively.

Our results showed that the adverse effect of heating on the spores (conidia) of *A. flavus* NRRL 5906 began at a temperature greater than 52 °C when the heat was applied for 2.5 and 5 min, resp., (Fig. 1) and that heating at 59 °C for at least 5 min is considered sufficient to inactivate spore germination. Generally, 5 min heating at 53 °C in combination with 0.75 kGy of gamma radiation resulted in complete inactivation of spores of *A. flavus* (Fig. 3). The fact that different strains of a species of fungus may vary in sensitivity to heat and irradiation combination treatment has been shown by other workers (SOMMER et al., 1964; MOYHUDDIN & SKOROPAD, 1975; WEBB et al., 1969).

From the economic point of view, lowering the radiation dose by the combination process is advantageous and desirable for the effective control of germinating spores of fungi. This type of in vitro studies will be relevant for products like fruits and vegetables which can be immersed directly in moderately warm water without any adverse effect on the plant tissue. It would, in this instance, be superfluous to immerse the product for more than 5 min in the warm water since no significant difference was found between the $D_{10}$ after 5 and 10 min heating at 53 °C (Table 1).

Our experimental results show that spores of *A. flavus* NRRL 5906 irradiated in suspension (Treatment A) were more radiation sensitive than those treated at different humidity conditions (Treatment B). Whilst a sublethal temperature of 53 °C applied for 5 min in combination with 0.75 kGy inactivated spores suspended in Tween solution, a dose of 4.0 kGy was required in combination with prior heating under high humidity conditions ($\geq$85% R. H.) at 60 °C for 30 min to achieve the same purpose. These findings are in agreement with the report of NYERGES-ROGRÜN (1975) whose test fungus was *Penicillium purpurogenum* Stoll, Strain No. 787.

We recorded a higher killing effect when the spores were heat-treated under high humidity ($\leq$85% R. H.) conditions before irradiation than when low humidity ($\leq$45% R. H.) prevailed during the heating prior to irradiation. Thermal inactivation of microorganisms is due to the denaturation of enzymes and other proteins. Perhaps the stress of heat on essential biological molecules of the spore is augmented by high humidity conditions. There is intensified diffusion of heat and moisture as well as increased target size due to thermal expansion of cell wall. It is well documented that in addition to the temperature and duration of treatment, humidity strikingly affects lethality of heat applied. Moist heat shows greater lethality for two reasons; i.e. enzymes are more readily coagulated and hydrated, and heat is transferred more readily in humid air (HAWKER et al., 1952). This sensitization renders the spores more vulnerable to irradiation affecting the nucleus of the spores. The damage to cellular DNA is one of the major factors responsible for radiation lethality. We cannot explain from our present data, the recovery of heated (60 °C for 30 min) and unirradiated spores after being at 80% R. H. for 8 days.

Studies of this nature, using moist heat (high humidity) to inactivate fungi on stored products in combination with gamma radiation will be more relevant to cereal grains and grain products which cannot be dipped into water and therefore furnish important information for direct application. The dose and heat requirements may however depend on the type of fungi and product and needs to be determined in a preliminary experiment.

*

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# MICROBIOLOGICAL STUDIES ON EGYPTIAN MARKET SALADS

ZAKIA A. HELMY, AFAF ABD-EL-BAKEY and Z. Y. DAW

Microbiology Department, Faculty of Agriculture, Cairo University, Cairo-Giza. Egypt

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Twenty samples of commercially prepared tehina and green vegetable salads as well as ten samples of mayonnaise salad were analysed. Bacteriological evaluation included determination of total plate counts, coliforms, faecal enterococci and salmonellae.

Green vegetable salad had the highest mean value of total viable counts ($8.0 \times 10^6$ per g), while the tehina salad had the lowest mean value count ($6.3 \times 10^4$ per g). Coliforms including faecal types were recovered in considerable number from the green vegetable and mayonnaise salads. Tehina salad contained large number of enterococcus organisms, they also occurred in considerable numbers in the green vegetable and mayonnaise salads. Salmonellae were not recovered from the green salad, while they were detected in 10 and 30% of the tehina and mayonnaise samples, respectively.

Effect of washing treatments on the bacterial count of the mixed green salad was also examined. Dipping the green vegetable in 0.3% potassium permanganate solution, washing with tap water or Rabso solution reduced the initial plate count (reduction rates: 98, 85 and 91% respectively).

**Keywords:** market salads, bacteriology, fresh vegetables

During the past few years, there has been an increased consumption of commercially prepared salads. Such foods generally are neither heated to kill pathogenic and spoilage organisms nor frozen to prolong their storage life.

No information concerning the microbial content of the commercially prepared salads in Egypt is available. However, in other foreign countries, limited number of research has been done to assess the safety of such foods (CHRISTIENSEN & KING, 1971; FOWLER et al., 1973; FOWLER & CLARK, 1975; HARRIS et al., 1975). Food poisoning and infection due to contaminated salad has been reported by MEYER and OXHOJ (1964); PETERSON (1964); ANON. (1976); FOSTER (1976).

From the most popular salads accepted by the consumer in Egypt, the mayonnaise, tehina and green vegetable salads are chosen for the present study.

Mayonnaise has been reported by SMITTLE (1977) to be resistant to most microbial spoilages but spoiled only by a few selected groups of microorganisms. Referring to the same author the salad if properly prepared will not support growth of pathogenic bacteria, and consequently it is of little public health concern.

Tehina salad is a local kind. During its preparation, it can be mishandled and become contaminated from the raw material or from equipment not properly cleaned. When the conditions, from the acidity and salt points of view, are favourable, microbial growth is expected and, occasionally tehina may be a vehicle of food poisoning.

Fresh vegetable salads generally include tomatoes, carrots, cucumbers, onion, lettuce in the presence of homogeneous mixture of salt, lemon juice and acetic acid. The microbial population of fresh vegetables reflects the location of origin (DOUGLAS, 1930), and thus, the presence of organisms which are usually associated with air, soil, water, sewage and the animal populations in the area can be expected. However, bacterial growth in such salads depends on their initial conditions.

The purpose of the present study, therefore, is to determine the microbial content of salads appearing on the retail market in an attempt to gain some insight into potential microbiological problems associated with these products. Special interest was given to organisms of pathogenic nature in fresh and stored salads, to detect reasons for their growth or inhibition.

Since the storability of foodstuffs depends, among other factors, on a low initial level of microbial population, an agent which reduces the numbers present may extend the shelf-life and also can suppress the growth of certain bacterial types. Thus consideration was also given to different washing and dipping methods applied for the mixed green salad to determine their effectiveness in reducing initial populations.

## 1. Materials and methods

Three products, mayonnaise, tehina and green vegetable salad were selected for this study. Mayonnaise is the semisolid emulsion of edible vegetable oil, vinegar, lemon juice, egg yolk, salt, mustard, paprika and other spices (FOOD AND DRUG ADMINISTRATION, 1975). Tehina is a popular salad in Egypt, made of tehina, a product from sesame mash, acetic acid and water, while the green vegetable salad is composed of lettuce, cucumber, pepper, onion and tomatoes, in addition to lemon juice and vinegar.

Twenty samples from each of tehina and green vegetable salads as well as 10 mayonnaise samples were purchased from different shops and restaurants in Cairo area. These were collected in sterilized containers.

A 50 g sample of each was weighed into a sterile 1 $dm^3$ blender cup and 450 $cm^3$ of sterile 0.01% peptone water, were added. The samples were blended for 3 min at high speed. Serial dilutions were prepared in peptone water for bacteriological analysis. The samples were examined for total plate counts using glucose lactose tomato juice agar medium and were incubated at 30 °C

for 3 days. For total and faecal coliform MacConkey broth medium was used and incubation at 37 °C for total coliforms and at 44.5 °C for faecal coliforms (COLIFORM SUBCOMMITTEE, 1949). For enterococcal counts, Bagg broth medium was used (HAJNA & PERRY, 1943). Presence of salmonellae was detected according to the procedure applied by GEORGALA and BOTHROYD (1965) and KHAN and MCCASKEY (1973). Isolates were biochemically identified according to the scheme applied by KAUFFMANN (1972) and JAWETS and co-workers (1976).

For studying the effect of storage temperature on the total plate counts of salad, two batches were adjusted to pH 4.5, 5.0 and 4.3 of each tehina, mayonnaise and green vegetable salads, resp. One batch was stored at 3 °C, while the other at 30 °C. Samples were examined at 0 h and after 1, 2, 3, 4, 6 and 7 days.

Effect of washing with 1. tap water, 2. detergent solution (5% Rabso), or 3. with 0.3% potassium permanganate, was also investigated. After treatments 2 and 3 the vegetables were thoroughly washed with tap water. Samples were taken before and after each treatment, and examined for total bacterial count, total and faecal coliform and enterococcal counts.

Samples were also chemically analysed for total chlorides according to JOFFEE (1942) and pH was measured using a pH meter.

## 2. Results and discussion

### *2.1. Mayonnaise salad*

As shown in Table 1, most of the samples showed high total plate counts, ranging from $10^5$–$10^8$ cells per g. This is generally attributed to contaminated ingredients mainly eggs and vegetables as well as to contaminated manufacturing equipment and unhygienic surroundings.

Total coliforms were recovered from all of the samples, but in relatively low numbers, however, two samples, showed a high contamination level of about $10^6$–$10^7$ cells per g.

Enterococcus types were found in considerable numbers in 9 out of the ten samples examined, while salmonellae were detected in 30% of the mayonnaise samples.

As from the chemical point of view, these salads were shown to be of acidic nature, the pH values ranged from 4.7 to 6.0, while their salt content averaged 0.6 to 1.4. Under these conditions in addition to other antagonistic factors that might be present in such salad, it can be said that the high initial microbial populations present resulted logically from contaminated ingredients rather than from the growth of organisms. In correlating the pH values with the microbial content of each sample, no relationship was found, a result which emphasizes the previous assumption.

Gram negative rods as well as the Gram positive spore formers constituted mostly the highest percentage of total isolates (Table 2). However, such organisms were likely to be contaminants from vegetable ingredients (FABIAN & ORLOFF, 1950), whole eggs, machines and utensils. The coccus forms, which seemed to include enterococci and staphylococci, as well as the non-sporogenic Gram positive rods, were detected in lesser percentages. Few samples showed yeast contaminants and as found previously (BAUMGART, 1965) was most commonly observed in freshly prepared mayonnaise.

Table 1

*The log numbers of total and differential cell counts, salt content and pH values of different mayonnaise salad samples*

| Samples No. | Log numbers per g of | | | | | NaCl(%) | pH |
|---|---|---|---|---|---|---|---|
| | Total viable counts | Total coliforms | Faecal coliforms | Enterococci | Salmonellae | | |
| 1 | 8.9 | 2.0 | — | 6.0 | — | 0.94 | 4.7 |
| 2 | 6.3 | 4.0 | 2.0 | 1.7 | ++ | 0.56 | 5.2 |
| 3 | 7.1 | 7.0 | 4.4 | 3.4 | — | 0.76 | 5.0 |
| 4 | 6.2 | 2.7 | 1.7 | 1.7 | ++ | 0.88 | 5.0 |
| 5 | 6.4 | 3.7 | — | 2.4 | — | 1.25 | 6.0 |
| 6 | 6.6 | 3.4 | 2.3 | 1.7 | — | 1.02 | 5.3 |
| 7 | 5.5 | 2.0 | — | — | — | 1.40 | 4.8 |
| 8 | 7.3 | 2.0 | 1.4 | 4.0 | ++ | 0.70 | 4.9 |
| 9 | 6.7 | 6.0 | 2.2 | 2.0 | — | 0.84 | 4.8 |
| 10 | 6.5 | 1.4 | — | 3.7 | — | 0.93 | 5.0 |
| Mean | 6.7 | 3.4 | 1.4 | 2.7 | | 0.925 | 5.07 |

Table 2

*Percentage distribution of bacterial groups isolated in mayonnaise samples*

| Samples No. | Total viable counts (log numbers per g) | Percentage distribution of | | | | |
|---|---|---|---|---|---|---|
| | | Gram negative rods | Gram positive sporeformers | Gram positive non-spore-formers | Gram positive cocci | Yeasts |
| 1 | 8.9 | — | — | 100 | — | — |
| 2 | 6.3 | 5.3 | 5.3 | — | 10.5 | 78.9 |
| 3 | 7.1 | 90.2 | 3.1 | — | 5.4 | 1.0 |
| 4 | 6.2 | 5.9 | 82.4 | — | 11.8 | — |
| 5 | 6.4 | 12.0 | 60.0 | 12.0 | 16.0 | — |
| 6 | 6.6 | 12.5 | 50.0 | 17.5 | 12.5 | 7.5 |
| 7 | 5.5 | — | 86.7 | 6.7 | 6.7 | — |
| 8 | 7.3 | — | 90.0 | — | — | 10.0 |
| 9 | 6.7 | 35.3 | 17.6 | 7.8 | 39.2 | — |
| 10 | 6.5 | 3.3 | 83.3 | 3.3 | 12.0 | — |
| Mean | 6.7 | 16.5 | 47.8 | 14.7 | 11.4 | 9.8 |

*2.2. Tehina salad*

Table 3 shows that from the twenty samples examined 18 samples had counts ranging from $1.4 \times 10^4$ to $4.0 \times 10^5$ org. per g. The lowest and highest counts recovered were $3.0 \times 10^3$ and $9.0 \times 10^6$, respectively.

Coliforms detected in 85% of the samples, ranged from less than 10 to nearly 10 000 cells per g. The faecal types, recovered from 50% of the positive samples, were detected in counts not higher than 100 cells per g.

Frequently, most of the microbial flora were of enterococcus type, and they were detected in nearly all of the tehina samples in relatively high numbers. Salmonellae, on the other hand, were recovered from only two samples.

A greater variation in the concentration of salt was observed between the samples, however, it seemed to have no effect on the bacterial population as it was within the limits tolerated by most organisms, on the other hand the pH level between 4.5–5.5 might inhibit the growth of many organisms in such salads.

Table 3

*Log numbers of total and differential cell counts, salt content and pH values of different fresh and stored tehina salad samples*

| Samples No. | Log numbers per g of: Total viable counts | | Total coliforms | | Faecal coliforms | | Enterococci | | Salmonellae | NaCl (%) | pH | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 h | 24 h | 0 h | 24 h | 0 h | 24 h | 0 h | 24 h | | | 0 h | 24 h |
| 1 | 5.1 | 5.1 | 2.4 | 1.2 | — | — | 1.7 | 4.4 | — | 0.80 | 4.9 | 4.5 |
| 2 | 4.8 | 5.0 | 2.0 | 0.6 | — | — | 4.0 | 5.0 | — | 1.06 | 4.8 | 5.0 |
| 3 | 4.7 | 4.7 | 1.0 | 3.4 | 1.0 | 1.0 | 3.7 | 6.2 | ++ | 1.54 | 5.0 | 5.0 |
| 4 | 5.4 | 4.6 | 3.9 | 5.0 | 2.0 | 2.0 | 2.4 | 1.4 | ++ | 3.09 | 5.5 | 5.3 |
| 5 | 4.9 | 5.0 | — | — | — | — | 5.0 | 4.0 | — | 0.84 | 4.2 | 4.3 |
| 6 | 4.5 | 6.4 | — | — | — | — | 3.2 | 3.7 | — | 0.99 | 4.3 | 4.5 |
| 7 | 4.7 | 4.7 | — | — | * | — | 4.7 | 6.0 | — | 0.92 | 4.5 | 4.7 |
| 8 | 3.5 | 4.3 | 3.4 | — | 2.0 | — | 2.4 | 4.0 | — | 1.10 | 4.4 | 4.4 |
| 9 | 4.4 | 4.2 | 4.0 | 1.7 | 2.0 | — | 4.4 | 5.7 | — | 0.91 | 4.8 | 4.3 |
| 10 | 4.3 | 5.5 | 1.4 | * | 1.2 | — | 1.4 | 4.3 | — | 1.95 | 4.6 | 4.4 |
| 11 | 5.2 | * | 3.3 | * | 0.9 | * | 6.0 | * | — | 0.73 | 5.0 | * |
| 12 | 5.6 | * | 1.0 | * | — | * | 0.6 | * | — | 1.6 | 4.7 | * |
| 13 | 4.3 | * | 1.0 | * | — | * | 4.0 | * | — | 1.31 | 4.9 | * |
| 14 | 5.3 | * | 3.0 | * | — | * | 5.1 | * | — | 1.61 | 5.0 | * |
| 15 | 4.2 | * | 3.0 | * | — | * | — | * | — | 1.90 | 4.0 | * |
| 16 | 4.4 | * | — | * | — | * | 0.6 | * | — | 0.88 | 4.9 | * |
| 17 | 4.2 | * | 2.0 | * | — | * | 2.7 | * | — | 0.88 | 4.8 | * |
| 18 | 4.6 | * | 2.7 | * | 1.4 | * | 1.0 | * | — | 4.38 | 4.9 | * |
| 19 | 7.0 | * | 2.0 | * | 1.0 | * | 6.3 | * | — | 1.90 | 4.7 | * |
| 20 | 5.4 | * | 2.4 | * | — | * | 0.6 | * | — | 2.05 | 4.7 | * |
| Mean | 4.8 | | 2.0 | | 0.6 | | 3.0 | | | 1.52 | 4.75 | |

* = not examined

Table 4

*tribution of each of the microbial groups in the different tehina samples*

| | Total viable count (log number per g) | Percentage distribution of | | | |
|---|---|---|---|---|---|
| | | Gram negative rods | Gram positive sporeformers | Gram positive non-sporeformers | Gram positive cocci |
| | 5.2 | 6.3 | 25.0 | 18.8 | 50.0 |
| | 5.6 | 60.0 | 20.0 | 10.0 | 10.0 |
| | 4.3 | 5.3 | 15.8 | 26.3 | 52.6 |
| 14 | 5.3 | 9.1 | 36.4 | 40.9 | 27.3 |
| 15 | 4.2 | 21.4 | 42.9 | 14.3 | 21.4 |
| 16 | 4.4 | 7.4 | 63.0 | 14.8 | 14.8 |
| 17 | 4.2 | 6.3 | 81.3 | 6.3 | 6.3 |
| 18 | 4.6 | 11.1 | 69.4 | 11.1 | 8.3 |
| 19 | 7.0 | 3.3 | 14.4 | 4.4 | 77.8 |
| 20 | 5.4 | 12.0 | 76.0 | — | 12.0 |
| Mean | 5.0 | 14.2 | 44.4 | 14.7 | 28.0 |
| Range | 4.2<br>7.0 | 5.3<br>60.0 | 14.4<br>81.3 | 0<br>40.9 | 6.3<br>77.8 |

When some of the samples examined were kept for 24 h at room temperature, no obvious increase in the total viable count was observed. However, the slight growth detected in four of them, showed an increase of similar magnitude in the enterococcus counts, thereby revealing that these conditions, including the pH level, may allow the growth of certain pathogenic indicator organisms. It was also found that spore formers were the most predominant contamination (Table 4). This was expected since they are soil organisms generally present on sesame seeds from which tehina is extracted. Gram negative rods, except in one sample, were the group found in the lowest number. The high incidence of this group in the one sample, was probably not due to coliform organisms as they were recovered in very small numbers, but to *Pseudomonas* type originating from either soil or water. The non-sporogenic gram positive rods were as plentiful as the coccus forms, but their number was lower than that of *Bacillus* type. These were probably of lactic acid types, streptococci, *Leuconostoc* and lactobacilli which are associated with plants and this could account for their number. However, sample 19, in which faecal streptococci were found, reflects generally unsanitary production methods and handling processes.

*2.3. Green vegetable salad*

The twenty samples of the green vegetable salads showed high microbial counts. The minimum and maximum values of total counts obtained were $2.9 \times 10^5$ and $7.3 \times 10^7$ org. per g, with a mean value of $2.1 \times 10^7$ org. per g

Table 5

*Log numbers of total and differential cell counts, salt content and pH values of different green vegetable salad samples*

| Samples No. | Log numbers per g of | | | | | NaCl (%) | pH |
|---|---|---|---|---|---|---|---|
| | Total viable counts | Total coliforms | Faecal coliforms | Enterococci | Salmonellae | | |
| 1 | 5.5 | 5.0 | 4.7 | 4.0 | — | 1.12 | 3.9 |
| 2 | 6.3 | 5.7 | 5.4 | 3.0 | — | 1.50 | 4.05 |
| 3 | 7.8 | 7.7 | 6.2 | 5.2 | — | 1.88 | 4.7 |
| 4 | 6.9 | 6.0 | 5.4 | 5.4 | — | 1.87 | 4.35 |
| 5 | 6.5 | 5.7 | 4.0 | 3.3 | — | 2.25 | 4.0 |
| 6 | 6.6 | 5.0 | 4.4 | 5.0 | — | 0.94 | 4.05 |
| 7 | 6.8 | 5.7 | 3.2 | 5.0 | — | 1.88 | 4.35 |
| 8 | 7.1 | 6.4 | 5.4 | 5.7 | — | 0.94 | 4.4 |
| 9 | 6.5 | 6.0 | 3.2 | 4.4 | — | 1.50 | 4.2 |
| 10 | 7.2 | 6.7 | 6.2 | 5.2 | — | 1.50 | 4.2 |
| 11 | 7.8 | 7.2 | 7.0 | 2.2 | — | 0.92 | 4.2 |
| 12 | 7.7 | 5.7 | 3.7 | 3.7 | — | 1.46 | 4.1 |
| 13 | 6.5 | 5.3 | 1.2 | 2.4 | — | 0.37 | 4.3 |
| 14 | 7.9 | 6.7 | 4.7 | 4.4 | — | 1.40 | 4.7 |
| 15 | 7.8 | 6.4 | 4.4 | 4.4 | — | 1.09 | 4.8 |
| 16 | 6.9 | 3.7 | 2.7 | 1.2 | — | 0.92 | 4.4 |
| 17 | 7.2 | 6.2 | 5.4 | 2.2 | — | 2.40 | 4.2 |
| 18 | 7.2 | 5.2 | 4.4 | 1.7 | — | 2.67 | 4.1 |
| 19 | 6.7 | 5.0 | 3.0 | 2.75 | — | 0.96 | 4.1 |
| 20 | 5.8 | 4.9 | 2.7 | 3.0 | — | 0.77 | 4.0 |
| Mean | 6.9 | 5.8 | 4.4 | 3.7 | — | 1.37 | 4.2 |

(Table 5). Coliforms constituted a considerable fraction of the total counts, ranging from $4.5 \times 10^3$ to $4.5 \times 10^7$ per g with a mean value of $4.1 \times 10^6$ per g. Many were shown to be of faecal types. Enterococci were recovered in high numbers, the mean value being $6.4 \times 10^4$ per g. Thus, bacteria found in salads belonged to a group of organisms usually regarded as pathogenic indicators. *Salmonella* was not recovered from any of the salad samples, this could be attributed to the high acidity in salads. The salt level of the different samples possibly does not play an effective role, since it was within the range tolerated since it was within the range tolerated by microorganisms.

The Gram negative rods were the most predominant type that was recovered from all of the samples examined, representing 70–100% of the total population except in one of the samples. Other groups frequently isolated in low numbers were the Gram positive sporeforming rods, followed by the coccus forms. Yeasts were recovered from 50% of the samples, while Gram positive non-sporeforming rods were obtained only from 30% of the salads (Table 6.)

Table 6

*Percentage distribution of bacterial groups isolated in green vegetable salad samples*

| Samples No. | Total viable counts (log numbers per g) | Percentage distribution of | | | | |
|---|---|---|---|---|---|---|
| | | Gram negative rods | Gram positive sporeformers | Gram positive non-spore-formers | Gram positive cocci | Yeasts |
| 11 | 7.8 | 98.3 | 1.7 | — | — | — |
| 12 | 7.7 | 100.0 | — | — | — | — |
| 13 | 6.5 | 93.7 | 3.1 | — | 3.1 | — |
| 14 | 7.9 | 93.2 | 1.4 | — | 5.5 | — |
| 15 | 7.8 | 91.5 | 1.5 | 3.0 | 3.0 | 1.0 |
| 16 | 6.9 | 1.4 | 98.6 | — | — | — |
| 17 | 7.2 | 82.5 | 1.3 | 5.0 | 3.8 | 6.3 |
| 18 | 7.2 | 90.0 | 1.3 | — | 2.0 | 6.6 |
| 19 | 6.7 | 90.4 | 3.8 | — | 3.8 | 1.9 |
| 20 | 5.8 | 70.0 | 8.2 | 10.0 | 6.7 | 5.0 |
| Mean | 7.2 | 81.1 | 12.1 | 1.8 | 2.8 | 2.1 |

As shown in Fig. 1, there is a positive relationship between the pH values of different samples and the total coliforms recovered, which probably means that the samples tested may have been stored for a time enough to initiate growth and multiplication of the organisms at a more suitable pH.

So, it can be concluded that mayonnaise and the green vegetable salads had the highest microbial content with considerable counts of coliforms and

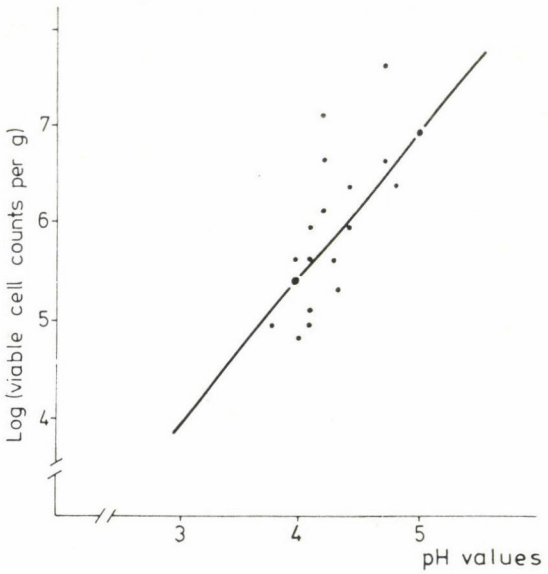


Fig. 1. Relationship between the pH values and the log coliform counts in salad mix samples. $Y = -0.68 + 1.52\,x$; correlation coefficient ($r$) = 0.528

enterococci. Although lesser total counts were recovered from the tehina salad, yet the conditions of production and handling allow contamination with certain unfavourable organisms such as enterococcus (Fig. 2).

To summarize the results, it can be said that, 95% of the green salad samples examined rendered total coliforms of $10^5$ organisms or more, while 40% of the mayonnaise samples showed lower counts ranging between 10–100 per g. Coliforms were recovered from only 25% of the tehina samples and the lowest count obtained was less than 10 cells per g. In addition, 45 and 30% of the green salad mixture and tehina samples, respectively, yielded enterococcus counts of $10^3$ per gor more and this level was represented by only 10% of the mayonnaise samples (Table 7).

Generally, it was found that the initial level of contamination of the different market salads was much higher than those previously reported for similar products (TANNER, 1944; FABIAN & WETHINGTON, 1950). Such observation reflected either bad production conditions, and/or growth initiation, providing that the mayonnaise samples were not fresh and probably have passed a considerable storage time under conditions allowing bacterial growth. In addition, soil residues on sesame seeds (JOPKE & RIDEY, 1968) and contamination by organisms during mashing and preparation processes because of inadequate cleaning of equipment, account for the variability obtained in the bacterial loads in samples of tehina salad. The high population encountered in green vegetable salads and the variation obtained between counts of samples, are in agreement with observations of SHAPIRO and HOLDER (1960),

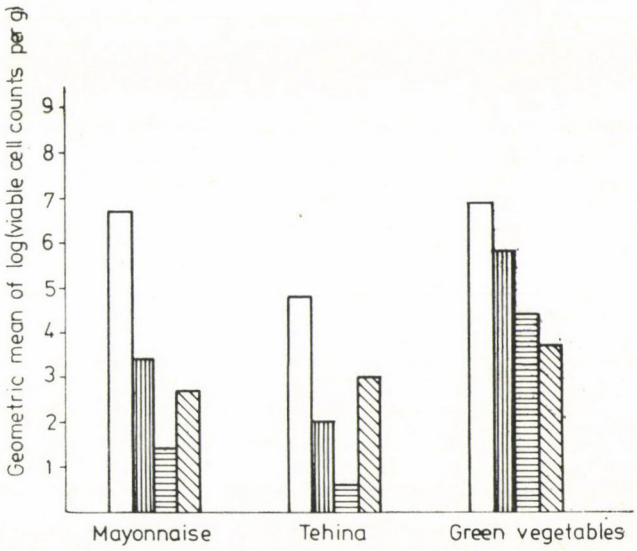


Fig. 2. Comparison of total and differential viable counts of the different salads. □: total counts; ▥: total coliforms; ▤: faecal coliforms; ▨: Enterococci

Table 7

*Frequencies of recovery and counts of the different groups isolated from the examined salads*

| Type of salad | Microorganisms | Percentage of salads with indicated cell counts per g | | | | |
|---|---|---|---|---|---|---|
| | | $<10$ | $10-10^2$ | $10^2-10^3$ | $10^3-10^4$ | $>10^4$ |
| Green vegetables | Total coliforms | — | — | — | 5 | 95 |
| | Faecal coliforms | — | 5 | 15 | 20 | 60 |
| | Enterococci | — | 10 | 30 | 15 | 45 |
| Tehina | Total coliforms | 35 | 25 | 20 | 20 | — |
| | Faecal coliforms | 75 | 25 | — | — | — |
| | Enterococci | 25 | 10 | 15 | 20 | 30 |
| Mayonnaise | Total coliforms | — | 40 | 18 | 20 | 20 |
| | Faecal coliforms | 40 | 30 | 20 | — | 10 |
| | Enterococci | 10 | 40 | 10 | 30 | 10 |

who reported that the finished product presented a greater surface for growth with an increased moisture content, as well as damaged tissues which function as an excellent substrate for growth.

Organisms isolated in high counts from salad samples were of hygienic significance, e.g. coliforms including the faecal types, and to a lesser extent the faecal enterococci. These types, have been reported to be normal inhabitants of plants. MUNDT (1970), FOWLER and CLARK (1975) and SAMISH and co-workers (1961) on the other hand, reported that microorganisms are present in the tissue of healthy tomatoes, while SPLITTSTOESSER (1970) stated that vegetables physically protected by pod or husk may possess counts as high as these, grown in direct contact with the environment. Pathogens from vegetable material was also documented in the literature (DUNLAP & WANG, 1961; BROWN & SEIDLER, 1973; GREEN et al., 1974). Other investigators have suspected egg-yolk and/or whole eggs for being a potential source of Salmonella contamination in mayonnaise (BOVRE, 1958; CORRETTI, 1963; BURDITT, 1965; KRAUSE, 1971).

Coliforms, are regarded as part of the normal microflora of the human intestinal tract and that of many animals, yet enteropathogenic serotypes have been reported to be implicated in human diarrheal diseases and food-poisoning outbreaks (SOJKA, 1973; SACK, 1975; KLIPSTEIN et al., 1977). Thus the probability that the high coliform counts recovered, especially from the green and mayonnaise salads, might implicate such serotypes, has to be considered and taken into account.

Salmonellae were recovered from 10 and 30% of the tehina and mayonnaise samples, respectively, of approximately pH 5.0. The green vegetable salad samples, being of higher acidity, were salmonellae free. The principal

potential source of salmonella contamination in mayonnaise is egg yolk and/or whole eggs (SMITTLE, 1977). However, the incidence of this type of organism in tehina possibly was a matter of mishandling and bad manufacturing practices.

*2.4. Effect of storage temperature on the bacterial flora of salad*

The initial as well as the total cell counts obtained in di[illegible] salads after different storage periods are illustrated in Fig. 3. When sala[illegible] stored at 30 °C, the initial log cell counts markedly increased, [illegible] doubled on the 2nd or on the 4th day of storage in case of mayonn[illegible] salad, respectively, then a quite rapid decline was observed. As regards green vegetable salad, a considerable growth was obtained during the first 24 h, such as ten-fold of the initial count, then, through the three following days the counts slightly decreased, after which a sharp death phase was observed. The great increase of counts was probably due to the presence of acid tolerant organisms, i.e., lactobacilli, yeasts, cocci and moulds. At 3 °C almost no visible growth was obtained, however the contaminants survived for more than 7 days, nearly at the same level as the initial value.

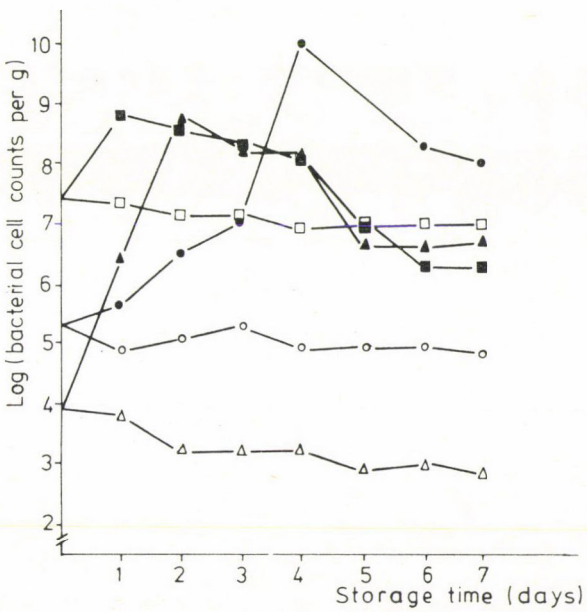


Fig. 3. Change of bacterial content as a function of storage temperature and time. —●—●—: mayonnaise 30 °C; —○—○—: mayonnaise 3 °C; —▲—▲—: tehina 30 °C; —△—△—: tehina 3 °C; —■—■—: green vegetable 30 °C; —□—□—: green vegetable 3 °C

*2.5. Effect of washing treatment on the bacterial content of the green salad*

As shown in Fig. 4, potassium permanganate was more effective in reducing the total bacterial flora than washing with either Rabso solution or tap water, reduction being 98.3, 90.6 and 85.0%, respectively. MAHMOUD and co-workers (1973) also reported that dipping in 0.3% of potassium permanganate resulted in the reduction by 98–100% of the microbial flora of vegetables.

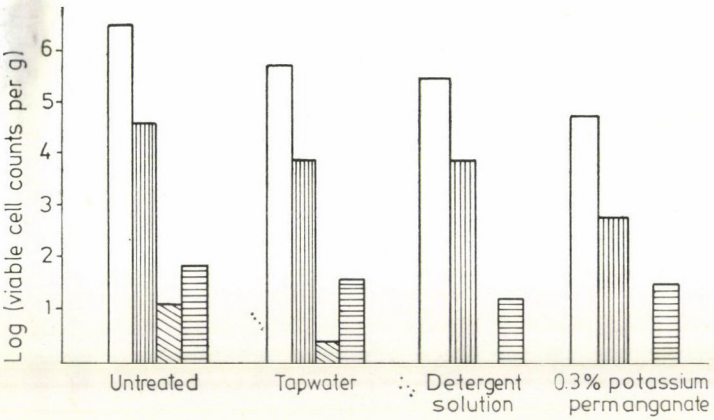


Fig. 4. Effect of different washing treatments on the total and differential viable counts in the green vegetable salad samples. □: total counts; ▥: total coliforms; ▨: faecal coliforms; ▤: Enterococci

In spite of the small numbers encountered for enterococci none of the washing treatments could free the salad from them. This is probably due to the fact that some of the used vegetables were injured and a few cells were distributed through the plant tissues, and thus escaped washing. On the other hand, evidence has been reported on the occurrence of bacteria within fresh healthy fruits and plants (SAMISH et al., 1961). Thus, it seems that enterococci might be present within the tissues of some of the applied vegetables.

So, it is quite evident that sanitary production and handling of salads, as well as proper storage conditions would possibly result in a good quality product, safe from the point of view of hygiene.

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# KINETIC ANALYSIS OF PECTINLYASE SYNTHESIS OF AN ASPERGILLUS STRAIN

KORNÉLIA ZETELAKI-HORVÁTH and NGUYEN XUAN THIEN

Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary

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Pectinlyase (PL) is able to decompose highly esterified pectins without the action of pectin esterase. In the course of the enzyme action, no methanol formation can be observed and the volatile ester content, responsible for the aroma of the various fruits, is not damaged. The use of endo-polygalacturonase and pectinesterase containing enzyme complex is not advantageous in the fruit juice industry, for the de-esterified pectin derivatives coagulate with the Ca-content of the fruit juices decreasing their stability.

**Keywords:** Growth kinetics, kinetics of product formation, pectinlyase synthesis

Pectinlyase is produced by fungi. Pectinlyase production of an *Aspergillus niger* strain was attempted to be increased by various mutagenic treatments (ZETELAKI-HORVÁTH et al., 1981) in our previous work. In the present work the growth and pectinlyase synthesis of a strain isolated after mutagenic treatment was kinetically analysed.

## 1. Materials and methods

### *1.1. Microorganism*

As test organism, an *Aspergillus niger* strain, (A-30) isolated after $NaN_3$ mutagenic treatment was used.

### *1.2. Media*

As the medium, an extract of dried sugar beet slices (cossettes: by-product of the sugar industry) was used. Extracts were concentrated in a vacuum evaporator, then they were adjusted to dry matter content of 1, 2, 4 and 6% with an Abbe-type refractometer (Zeiss, Jena). The values of the dry matter contents are referred to later as concentration (refr. %) of carbon source (or extract). The dry matter content originating from the salts added (cca 85%) was not taken into account in the terminology adapted in this

article. One $dm^3$ of the above extracts were complemented with the followings: malt sprout extract 50 $cm^3$, $(NH_4)_2SO_4$ 6 g, $KH_2PO_4$ 0.6 g, $MgSO_4$ 0.05 g, and trace elements. The pH was adjusted to 5.5.

*1.3. Cultivation*

Cultivation was carried out in 10 $dm^3$ glass fermentors with a working volume of 6.6 $dm^3$. The rate of agitation was 460 r.p.m. while the rate of aeration 1.5 $dm^3\,dm^{-3}\,min^{-1}$, resulting in an Oxygen Transfer Rate (OTR) of 60 mmol $O_2$ $dm^{-3}\,h^{-1}$.

One hundred $cm^3$ samples were taken in every six hours. Mycelia were isolated from the samples by filtration. Residues of the media were removed by thorough washing. Mycelia were dried at 105 °C to constant weight.

*1.4. Enzyme activity*

Pectinlyase (PL) activity was determined from the culture filtrate, by the method of ALBERSHEIM and co-workers (1960), modified according to the results of our measurements (ZETELAKI-HORVÁTH, 1982). Pectinlyase activity was expressed as $\mu$mol unsaturated galacturonic acid liberated by 1 $cm^3$ culture filtrate in a minute.

*1.5. Kinetic analysis*

Growth and pectinlyase synthesis of the selected strain was kinetically analysed by the method of KONO and ASAI (1968).

*1.6. Mathematical statistical calculations*

Variation coefficients of the kinetic constants of five fermentations, carried out under identical conditions, were calculated.

## 2. Results

*2.1. Growth and enzyme production*

The growth of the A-30 *Aspergillus niger* was investigated in media of various concentrations of sugar beet extract. With the increase of the extract concentration, a prolongation of the transient phase of growth was found. Maximum mycelial yields increased from 4.3 to 5.8 and 8.3 g $dm^{-3}$ with the increase in the extract concentration from 1 to 2 and 4%, respectively. No further significant increase in the mycelium yield was obtained when a 6%

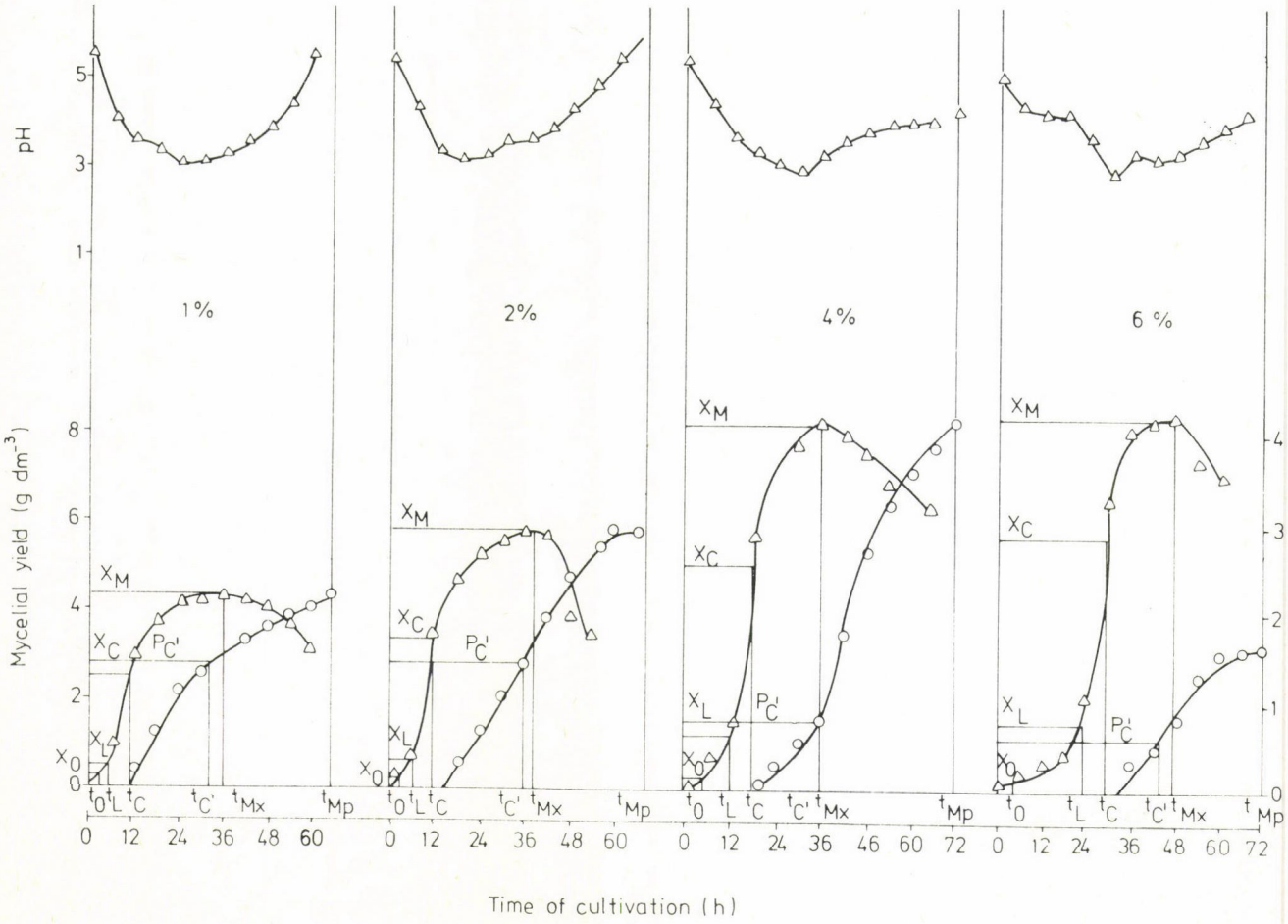


Fig. 1. Growth and pectinlyase synthesis of *Aspergillus niger* as a function of the time of cultivation in media of various extract concentrations

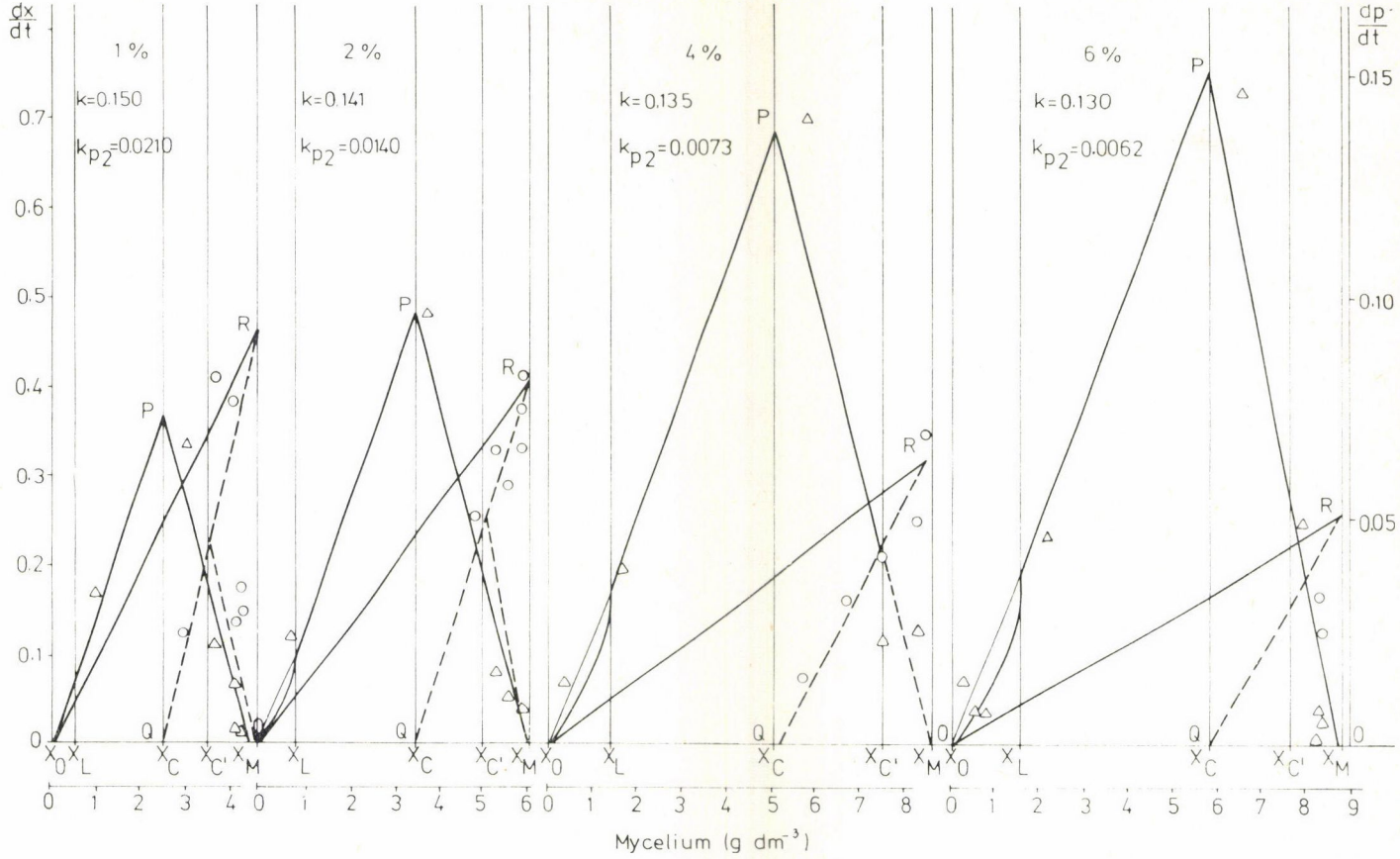


Fig. 2. Rate of growth and pectinlyase formation of *Aspergillus niger* as a function of mycelial concentration in media of various extract concentrations

sugar beet medium was used (Fig. 1). Maximum growth was attained in the 36 and 48 hour culture at an extract concentration of 1, 2, 4 and 6%, respectively.

The pH values of the culture filtrate showed a decreasing tendency until the beginning of the autolysis of the cells (Fig. 1).

Pectinlyase production started only in the declining phase of the cultures. Enzyme concentration in the culture filtrate increased with the increase of the concentration of the sugar beet extract from 1 to 4%, while in the medium of 6% extract concentration, pectinlyase activity of the culture filtrate was lower than that in the 1% extract. Maximum pectinlyase yield was detected in the 60 hour culture with the exception of the 4% medium, where maximum PL yield occurred in the 72 hour culture.

A simple four phase growth of the culture has been found when the rate of growth of the culture was plotted against mycelium concentration (Fig. 2).

The rate of growth ($k$: which is represented by the slope of the exponential phase) decreased with the increase of the extract concentration from 1 to 6%, respectively, as a consequence of the increasing mycelium yield in the exponential phase and the increased value of the critical mycelium concentration ($x_c$) (Fig. 2).

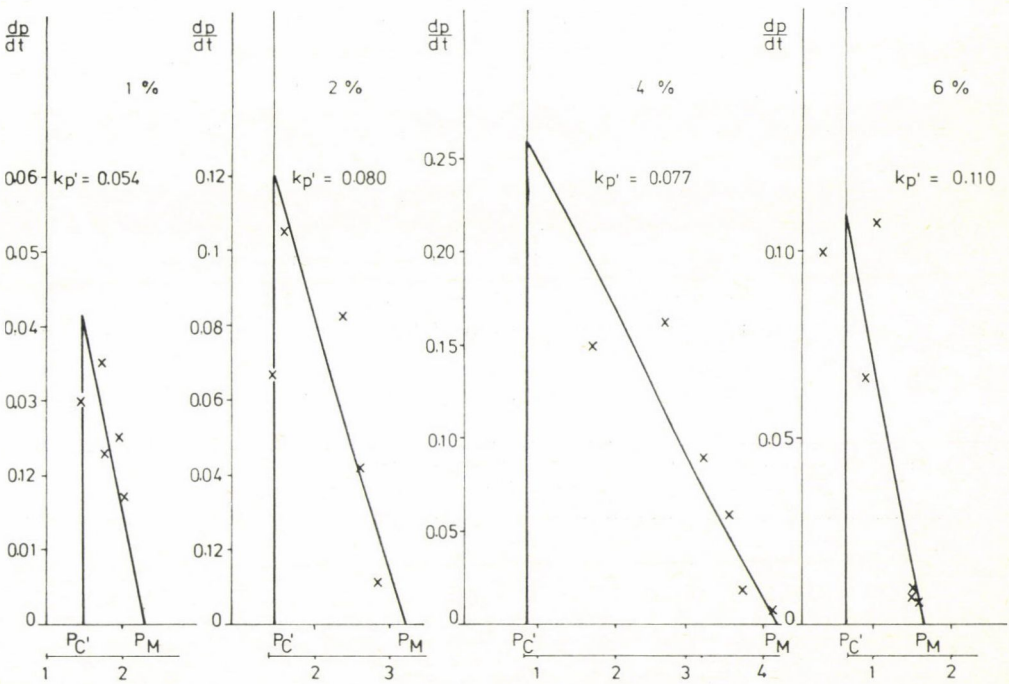


Fig. 3. The rate of final pectinlyase formation of *Aspergillus niger* as a function of the pectinlyase concentration

Pectinlyase formation of the investigated *Aspergillus niger* strain proved to be non-growth related, as no enzyme production could be detected in the phase of intensive growth (exponential growth phase), and the value of the growth-related production rate constant ($k_{p1}$) proved to be zero.

The production rate constants of the resting cells ($k_{p2}$) showed the highest value (0.0210 $h^{-1}$) in the culture, incubated in a medium of 1% sugar beet extract. The values of $k_{p2}$ in the media of 2, 3 and 4% extract concentrations were as follows: 0.0140, 0.0073 and 0.0062, respectively.

Product formation of the cultures could be described only with the use of another production rate constant which is $k_{p'}$, representing the final formation (Fig. 3).

Value of $k_{p'}$ proved to be the highest (0.11 $h^{-1}$) in the medium of highest extract concentration. Its values were similar (0.08 and 0.077 $h^{-1}$) in the media of 2 and 4% extract concentration, respectively, while the lowest value was measured in the medium of 1% extract.

## 3. Conclusions

In contrast to our previous work (ZETELAKI-HORVÁTH & BÉKÁSSY-MOLNÁR, 1973), no diauxic growth of the culture was obtained in the medium of sugar beet extract.

This might be the result of the introduction in the sugar factories of a new technology of higher extraction as a result of which the sugar content of the extract became very low.

The highest mycelium yield ($x_M = 8.4$ g $dm^{-3}$) was obtained in the medium of highest concentration (6%), but no significant difference was observed between the $x_M$ values in media of 6 and 4% extract (Table 1).

The maximum pectinlyase yield ($p_M = 4.24$ μmol galacturonic acid $min^{-1}\ cm^{-3}$) was obtained in the medium of 3% extract, which proved to be

Table 1

*Kinetic constants of the growth of* Aspergillus niger *grown in sugar beet extract media* (cultivation: 10 $dm^3$ glass fermentors; r.p.m.: 460; aeration: 1.0 $cm^3\ cm^{-3}\ h^{-1}$; temperature: 32 °C)

| Medium conc. (%) | $t_0$ | $t_L$ | $t_C$ | $t_M$ | $x_O$ | $x_L$ | $x_C$ | $x_{C'}$ | $x_M$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 3 | 6 | 11.0 | 36 | 0.2 | 0.5 | 2.5 | 3.5 | 4.3 |
| 2 | 3 | 6 | 11.5 | 36 | 0.2 | 0.7 | 3.4 | 5.0 | 5.8 |
| 4 | 3 | 11 | 17.0 | 36 | 0.2 | 1.4 | 5.1 | 7.5 | 8.3 |
| 6 | 3 | 23 | 30.0 | 48 | 0.2 | 1.6 | 5.8 | 7.6 | 8.4 |

Medium conc.: concentration of the extract of the dried sugar beet slices

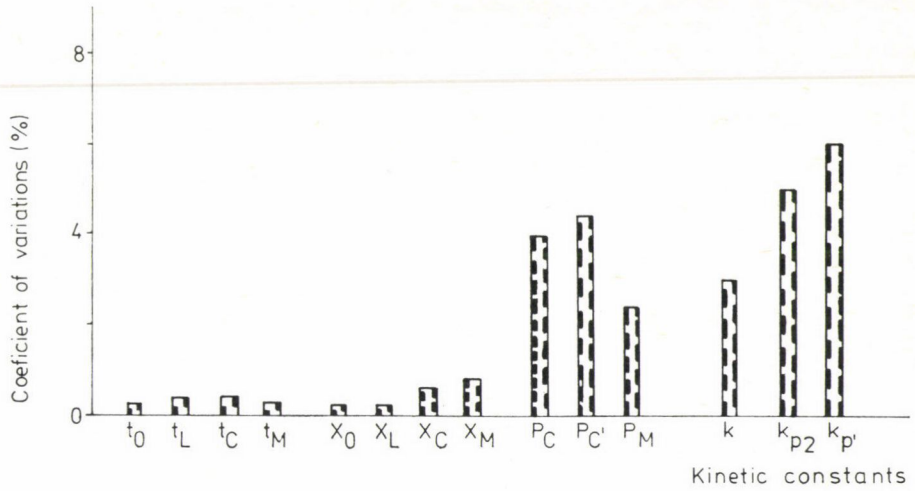


Fig. 4. Coefficient of variation of the kinetic constants of 5 cultures, incubated under identical conditions

very highly significantly higher than those obtained in media of other extract concentrations (Table 2). The lowest pectinlyase yield was obtained in media of highest extract concentration, and this can be the result of some substrate inhibition.

Pectinlyase production of the investigated *Aspergillus niger* strain belonged to the third type of product formation of KONO and ASAI (1969), for pectinlyase was synthesized only by the non-growing cells, when the values

Table 2

*Kinetic constants of the pectinlyase formation of* Aspergillus niger *grown in sugar beet extract media*
(cultivation: 10 $dm^3$ glass fermentors; r.p.m.: 460; aeration: 1.0 $cm^3\ cm^{-3}\ h^{-1}$; temperature: 32 °C)

| Medium conc. (%) | $k$ | $k_{p1}$ | $k_{p2}$ | $k_{p'}$ | $p_C$ | $p_{C'}$ | $p_M$ | $t_{p'}$ | $t_{Mp}$ |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.150 | 0 | 0.0210 | 0.054 | 0 | 1.40 | 2.20 | 33.0 | 66 |
| 2 | 0.141 | 0 | 0.0140 | 0.080 | 0 | 1.45 | 2.95 | 36.0 | 60 |
| 4 | 0.135 | 0 | 0.0073 | 0.077 | 0 | 0.85 | 4.24 | 35.5 | 72 |
| 6 | 0.130 | 0 | 0.0062 | 0.110 | 0 | 0.60 | 1.60 | 45.0 | 72 |

Medium conc.: concentration of the extract of the dried sugar beet slices

of $k_{p1}$ were zero while the values of the production rate constants ($k_{p2}$) of the non-growing cells proved to be positive.

To investigate the coefficients of variation of the kinetic constants, five fermentations were carried out under identical conditions. Among the kinetic constants, highest values of the coefficients of variation (4—6%) were obtained in the case of constants, concerning the product formation ($p_C$, $p_{C'}$, $p_M$ and $k$, $k_{p2}$, $k_{p'}$, resp.) (Fig. 4).

*Nomenclature*

$k$ growth rate constant ($h^{-1}$)
$k_{p1}$ production rate constant of the growing cells ($h^{-1}$)
$k_{p2}$ production rate constant of the non-growing cells ($h^{-1}$)
$k_{p'}$ production rate constant of final product formation ($h^{-1}$)

*Subscripts*

$O$ refers to the boundary of an induction phase and a transient phase
$L$ refers to the boundary of a transient phase and an exponential growth phase
$C$ refers to the critical point, that is the boundary of an exponential growth phase and a declining growth phase
$M$ refers to theoretical maximum value of cell and product concentration, respectively.

The slopes of $\bar{O}\bar{P}$, $\bar{O}\bar{Q}$ and $\bar{O}\bar{R}$ represent $k$, $k_{p1}$ and $k_{p2}$, respectively.

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# RELATIONSHIP BETWEEN PIGMENT CONTENT, PEROXIDASE ACTIVITY AND SUGAR COMPOSITION OF RED PEPPER *(CAPSICUM ANNUUM* L.)

## I. INFLUENCE OF CULTIVAR, DRYING METHOD AND A RIPENING ACCELERATOR

LILLY VÁMOS-VIGYÁZÓ[a], MÁRIA POLACSEK-RÁCZ[a], KATALIN SCHMIDT[a], ILDIKÓ JOÓ-FARKAS[a], MAGDA P. PAULI[b], GY. HORVÁTH[b], K. KISS[b] and L. HORVÁTH[a]

[a] Central Food Research Institute, H-1022, Budapest, Herman Ottó út 15. Hungary
[b] County Station for Animal Hygiene and Food Control, H-6001, Kecskemét, Széchenyi krt. 29. Hungary

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In order to acquire a better knowledge of the causes of paprika discolouration occurring during industrial scale processing, two parameters were studied which might be related to the phenomenon. One of them is sugar composition which might be the source of non-enzymatic browning, the other one is peroxidase (POD) activity. According to data from the literature, this enzyme is capable to degrade carotenoids and — being extremely heat stable — does not get inactivated during industrial processing (drying, grinding) of seasoning paprika.

Beside determination of peroxidase activity and glucose, fructose and sucrose contents by selective enzymatic methods, analyses comprised visual and instrumental colour estimation and/or determination of pigment content.

Variations in these characteristics were followed in samples of different cultivars during ripening and after-ripening. The effects of the application of a ripening accelerator as well as of the method of drying were studied in model experiments.

Some of the results obtained were consistent with data of the literature. The main findings not reported by others so far are as follows.

In the green state the dominating sugar in paprika pericarp is glucose, in the "smoky" state the ratio of glucose to fructose is about 1 : 1, in the red state fructose becomes the prevailing sugar. Turning red of the pods is connected to a drop in peroxidase activity. After-ripening brings about a further decrease in POD activity and also in sugar, mainly in glucose content.

Drying of the pods at room temperature results in a decrease in POD activity of about 50%, irrespectively of the value found in the fresh state. This latter is subject to strong varietal and yearly variations. Residual enzyme activity in dry pods was not found to affect pigment content. All samples dried at room temperature under after-ripening contained sucrose, often absent from ripe pods.

Freeze-drying applied right after picking is not suited to yield a high-quality product as it blocks biological processes at a too early stage of ripening, when both peroxidase activity and sugar content are still high.

The application of an ethephon-type ripening accelerator known to retard the formation of pigments, in particular the red ones, brings about a shift in the glucose–fructose ratio towards the latter. These effects cannot be reversed by a 21-day after-ripening period of the pods on the plant. A delay in sucrose accumulation during this period is another phenomenon observed in paprika treated with the ripening accelerator.

**Keywords:** Red pepper, pigments, peroxidase activity, cultivars, agrotechnics

Red pepper is a fundamental condiment of foods and also an important export article of Hungary. Changes in agrotechnics and the introduction of large scale processing technologies have, however, impaired its quality. Research work aimed at ensuring optimum colour and satisfactory colour stability of ground red pepper under the given conditions of cultivation and processing is being carried on in a number of institutions in Hungary. The present paper reports on investigations into the changes, during cultivation and processing, in some characteristics that might have a bearing on the colour and colour stability of paprika. Two of these characteristics are sugar composition and content, respectively, the changes of which might indicate the occurrence of non-enzymatic browning reactions. Peroxidase activity is another one. This enzyme might have a double function: it has been reported to be a catalyst of carotenoid bleaching reactions (KANNER et al., 1977), on one hand, and — being extremely thermostable — losses of its activity might indicate heat damage of colouring matter taking place during the drying process, on the other.

The investigations into the characteristics mentioned have been carried on since 1977 and comprise determination of the concentrations of the sugar components (glucose, fructose, sucrose) and of soluble peroxidase activity in samples of some cultivars grown in the Kalocsa region (South Hungary), one of the two important paprika-growing areas of the country. The assays were performed with fresh and after-ripened samples as well as with samples dried in the laboratory by different methods (heat treatment, freeze-drying and drying at room temperature under after-ripening). The effects of a ripening accelerator were studied as well. Considering the results of preliminary investigations (POLACSEK-RÁCZ et al., 1981a) analyses were restricted to the skin (pericarp) of the paprika fruit. Attempts were made to relate the results obtained to the colour determined by visual and instrumental methods and in some instances, to pigment and moisture contents.

## 1. Materials and methods

### *1.1. The paprika cultivars*

In the year 1980 samples of three cultivars (Sz 20, KCsG 631 and KM 622) were analyzed in the fresh state as well as after drying *a* at room temperature under after-ripening, *b* in an oven (80 °C) until reaching 7% moisture content and *c* by freeze-drying in a laboratory scale equipment.

In the year 1981 the cultivar KM 622 was used to study the effect of an ethephon-type ripening accelerator, Flordimex (VEB Chemie-Kombinat, Bitterfeld, GDR) applied by spraying at dose levels of 1 $dm^3$ per ha.

Some characteristics of the cultivars applied in the experiments are given below (SOMOS, 1981; SZÜCS, 1975).

Sz 20 is a cultivar of continuous growth with hanging pods. It has high solids and pigment contents, lends itself to machine picking and stores well. Fully ripe pods are dark red.

KCsG 631 is a quickly ripening cultivar with hanging pods. It lends itself to machine harvest and, owing to its high solids content, can be stored for a longer period without losses. It is of medium pigment content. Fully ripe pods are dark red.

KM 622 is one of the earliest ripening cultivars with pods turned upward on rigid stalks. This is favourable from the aspect of pigment formation, but unfavourable from that of picking. Fully ripe pods are dark red.

### *1.2. Methods*

*1.2.1. Determination of moisture content.* The skin of the fresh pods was separated from the veins and seeds, that of the dried pods from the seeds, was minced and ground, respectively, and dried in an oven at 70 °C till reaching constant mass. All the analytical data will be related to solids content.

*1.2.2. Sugar determinations.* Glucose, fructose and sucrose concentrations were determined in the water extracts of paprika pericarp separated from the rest of the fruit (POLACSEK-RÁCZ et al., 1981b) using enzymatic methods as described in Boehringer's leaflet (ANON, 1980). Analyses were performed in 2 replicates. The relative standard deviation of the methods was $<5\%$.

*1.2.3. Assay of peroxidase activity.* Peroxidase (POD) activity was assayed in extracts of fresh minced or dry ground paprika skin. The extraction was carried out with 0.2 mol $dm^{-3}$ acetate buffer, pH 5.0. POD activity was measured by a simplified version of the method of MIHÁLYI and VÁMOS-VIGYÁZÓ (1975). The simplification consisted in using a single reaction mixture and a single blank (both in triplicate) and reading the changes in optical density (OD) of the reaction mixture against the blank at regular intervals instead of using, for every reading, a separate reaction mixture and blank, respectively (as necessary with homogenates). The value of $10^{-3} \Delta$OD per min was considered unit activity ($U$) and was related to 1 g of solids content.

*1.2.4. Measurement of the colour.* The colour of ground paprika was measured in a Momcolor tristimulus device (Magyar Optikai Művek, Budapest) against a white standard. The differences in colour ($\Delta E$) and in hue ($\Delta H$) as compared to the sample of the best visual colour were calculated using a computer programme developed at the Division of Food Biophysics at the Central Food Research Institute, Budapest.

*1.2.5. Visual colour estimation.* Visual colour testing was carried out by a panel of three. A serial number was allotted by each one of the judges to

the 1, 2, . . ., $n$ samples, 1 standing for the best, $n$ for the worst colouration. The colour of a given sample was characterized by the mean of the three judges' classifications.

*1.2.6. Determination of pigment concentration.* Total pigment content was determined according to HUNGARIAN STANDARD (1976), a spectrophotometric method based on benzene extraction of dehydrated paprika powder.

The percentage of red pigments was assayed essentially by the method of BARANYAI and SZABOLCS (1976) based on differential spectrophotometry of the benzene extract and the red polyene ketones thereof as reduced by sodium borohydride.

*1.2.7. Application of the ripening accelerator and sampling of the treated parcels.* The ripening accelerator was applied on two experimental parcels. On one of these the majority of the pods had turned red by the time of spraying, the other one was sprayed before reaching this stage. These parcels will be referred to, hereafter, as "treated in time" ($F_t$) and "treated too early" ($F_e$), respectively. A third, unsprayed parcel served as reference.

Samples were picked 1 and 21 days after Flordimex treatment and were analyzed in the fresh state as well as after drying at room temperature and at 80 °C, respectively. Samples for freeze-drying were taken 1, 2, 3, 5, 10 and 21 days after spraying as — according to preliminary experiments (POLACSEK-RÁCZ et al., 1981a) — immediate freeze-drying of paprika sprayed with the ripening accelerator lead to extreme discolouration and high POD activity.

## 2. Results

*2.1. Comparison of the characteristics studied as found in samples of different cultivars in the fresh state and after drying by different methods*

The POD activity values found in the pericarps of the fresh and processed samples of the cultivars Sz 20, KCsG 631 and KM 622 are shown in Fig. 1.

The Figure shows POD activity to vary considerably with cultivars and drying methods. Activity in the fresh sample of the cultivar Sz 20 was found to be 7.5 and 5.5 times that of the cultivars KCsG 631 and KM 622, respectively. The samples dried at room temperature had enzyme activities about half those established in the fresh state. In the samples dried at an elevated temperature activities were negligible. The highest activities were found in the freeze-dried samples. Their values were higher, by 26%, 107% and 34% than those established for the fresh samples of the cultivars Sz 20, KCsG 631 and KM 622, respectively.

Figure 2 shows the sugar content and composition of the same samples.

The results show, in some respects, trends similar to those obtained for POD activities: freeze-dried samples had the highest and those dried at

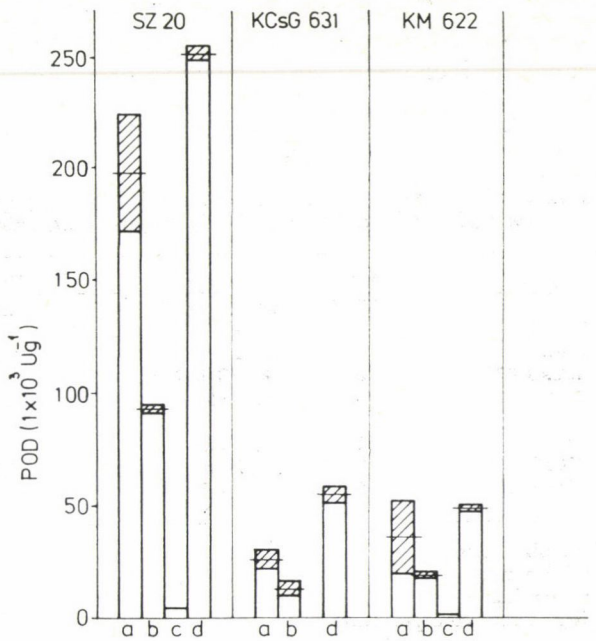


Fig. 1. Influence of cultivar and drying procedure on the peroxidase (POD) activity of red pepper. Cultivars: Sz 20, KCsG 631 and KM 622. State of samples: *a*: fresh; *b*: dried at room temperature, in strings; *c*: dried at 80 °C in a laboratory oven; *d*: freeze-dried. Values are related to solids content. The upper and lower ends of the shaded parts of the histograms represent the two values measured, the horizontal bars their means

elevated temperature the lowest sugar content, 25–30% below the levels found in the fresh samples. In two out of the three cultivars (KCsG 631 and KM 622) sugar contents increased during drying at room temperature (after-ripening). The increase was due to the formation of sucrose. This sugar appeared also in the third sample only during after-ripening. Drying at elevated temperature not only decreased sugar content, but modified its composition as well: the percentage ratio of glucose dropped from the values found in the rest of the samples, i.e. 27–37% for Sz 20, 29–39% for KCsG 631 and 24–34% for KM 622 to 17%, 21% and 18%, respectively. The ratio of glucose to fructose at the same time, considerably shifted towards the latter.

The results of visual and instrumental colour estimations of the samples dried at room temperature and of freeze-dried samples are summarized in Table 1.

The samples dried at 80 °C turned brown during the drying process and, therefore, have not been listed in the Table.

The samples dried at room temperature were of a better colour, a more reddish shade than the freeze-dried ones of the corresponding cultivars. Out

of the samples dried at room temperature that of the cultivar Sz 20 was found to have the best colour. It may be interesting to note that the freeze-dried sample of the same cultivar was rated worst both by visual and instrumental estimation.

Table 1

*Results of visual and instrumental colour estimation of paprika samples picked in 1980 and dried by different methods*

| Method of drying | Cultivar | Visual rating | Instrumental estimation | | |
|---|---|---|---|---|---|
| | | | reference sample | $\Delta E$ | $\Delta H$ |
| | Sz-20 | 6 | Sz 20 *d* | 17.17 | 11.04 |
| *b* | KCsG 631 | 5 | KCsG 631 *d* | 5.24 | 4.74 |
| | KM 622 | 4 | KM 622 *d* | 5.02 | 3.84 |
| | Sz 200 | 1 | Sz 20 *d* | 0 | 0 |
| *d* | KCsG 631 | 3 | Sz 20 *d* | 8.52 | 5.88 |
| | KM 622 | 2 | Sz 20 *d* | 4.64 | 2.62 |

$\Delta E$ = colour difference
$\Delta H$ = hue difference
*b* = freeze-drying
*d* = drying at room temperature

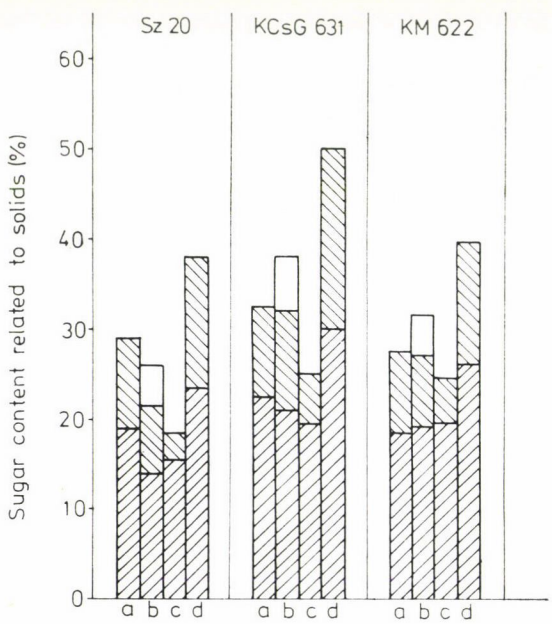


Fig. 2. Influence of cultivar and drying procedure on sugar content and composition of red pepper. □: sucrose; ▨: glucose; ▧: fructose. For the rest of symbols and explanations see Fig. 1

*2.2. Influence of the state of maturity reached on applying the ripeness accelerator as well as of the length of subsequent after-ripening on the characteristics studied in fresh and dried samples of the cultivar KM 622*

The state of ripeness of paprika, i.e. the ratio of green–smoky–red pods in the experimental and control parcels at the time of Flordimex treatment is shown in Fig. 3.

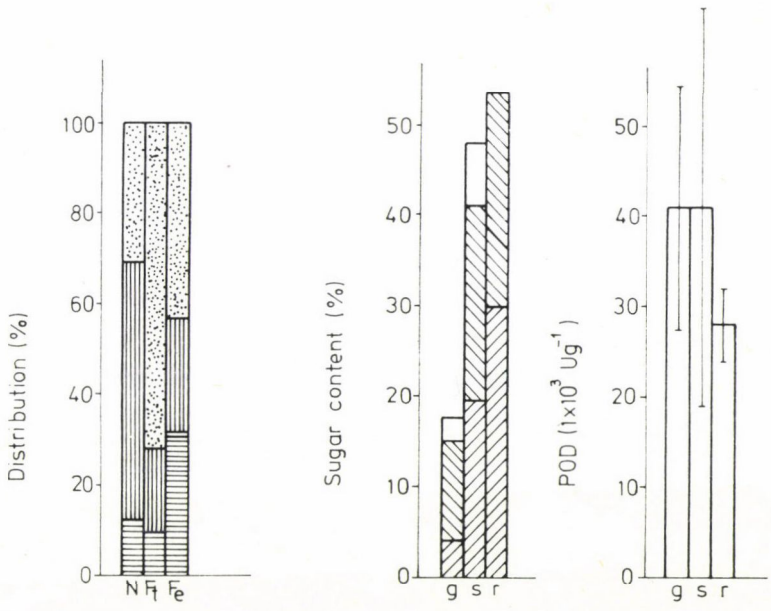


Fig. 3. The percentage ratio of green (▤), smoky (▥) and red (▦) pods on the parcels at spraying with the ripening accelerator as well as the concentrations of fructose (▨), glucose (▨) and sucrose(□ w per w), and the values of peroxidase (POD) activity in the pods of above colourations. The ripening accelerator Flordimex was sprayed at a level of 1 $dm^3$ $ha^{-1}$. *N*: unsprayed control parcel; $F_t$: parcel sprayed in time (after about 80% of the pods had turned red); $F_e$: parcel sprayed too early (before reaching the state of ripeness mentioned): *g*, *s* and *r* stand for green, smoky and red, resp. Cultivar: KM 622. Sugar content and POD activity are related to solids content. The vertical bars represent standard deviations. For the sugar components the variation coefficient (v) $< 5\%$

The Figure shows also the sugar content and composition as well as the POD activity in the pods of different colouration.

At the time of Flordimex application the crops on the three parcels were in different states of maturity. As a matter of course, pods were most ripe on the parcel to be sprayed in time. However, there was a difference in maturity between the pods on the other two parcels, too. In the pods from the control parcel the smoky stage was prevailing, while in those from the

parcel to be sprayed too early the three stages were present in nearly equal ratios. As can be seen from the Figure, this involves differences in initial sugar content and composition as well as in POD activity.

During the transition from the green to the smoky state, total sugar content increased nearly threefold, glucose and sucrose contents were more than doubled and fructose concentration rose nearly fivefold. During the transition from the smoky to the red state, fructose and total sugar contents showed a further increase of 53% and 15%, respectively, as related to the values found in smoky pods. Glucose content remained practically constant and sucrose content disappeared. The ratios of glucose to fructose were, at the three stages of ripening, in the order mentioned 72 : 28, 54 : 46, 44 : 56.

POD activity was similar in the green and smoky pods, and lower by about 30% in the red ones. However, owing to extremely high standard deviations, this decrease was not significant ($P > 0.5\%$).

The influence of the time elapsed from Flordimex treatment till picking, on POD activity and sugar composition as well as on sugar content, of fresh paprika and samples dried at room temperature and at 80 °C, respectively, is shown in Fig. 4. Out of the fresh samples (*a*) those treated with Flordimex had higher POD activities. From the samples dried at room temperature (*b*) the highest POD activity was found, after one day of after-ripening on the plant, in the untreated paprika. After 21 days of after-ripening, the increasing order was: untreated ($N$), treated in time ($F_t$) and treated too early ($F_e$). It might be of interest that in the samples treated with the ripening accelerator, longer after-ripening before drying lead to a higher, while in the untreated sample it leads to a lower enzyme activity. The samples dried at 80 °C (*c*) indicated, in the first place, the damaging effect of heat treatment. The highest POD activity was observed, also in this case, in the sample picked 21 days after too early Flordimex-treatment.

Sugar content of the fresh sample treated in time with the ripening accelerator was about 50% higher than in the other two samples. This fact as well as the lower relative glucose content indicate a more mature state. In contrast to the results obtained with the cultivar KM 622 in the preceding year (Fig. 2), drying at room temperature brought about a considerable decrease in sugar content (irrespectively of Flordimex treatment).

The effect of after-ripening on sugar content was similar in tendency, although less marked than on POD activity. In the majority of samples heat drying further decreased sugar content as compared to drying at room temperature. Furthermore, sucrose content disappeared and glucose content decreased, too. These findings are consistent with those obtained in the preceding year.

The changes occurring in POD activity during after-ripening as found in the freeze-dried samples are shown in Fig. 5.

The considerable increase in POD activity during the first five days of after-ripening of all three samples indicates that, when applying the ripening accelerator, the crops were still unripe. In later periods of after-ripening POD activities did not increase further, in sample $F_e$ even a decrease occurred.

In the after-ripening phase following the application of the ripening accelerator, the total sugar content of the freeze-dried samples slightly decreased (Fig. 6).

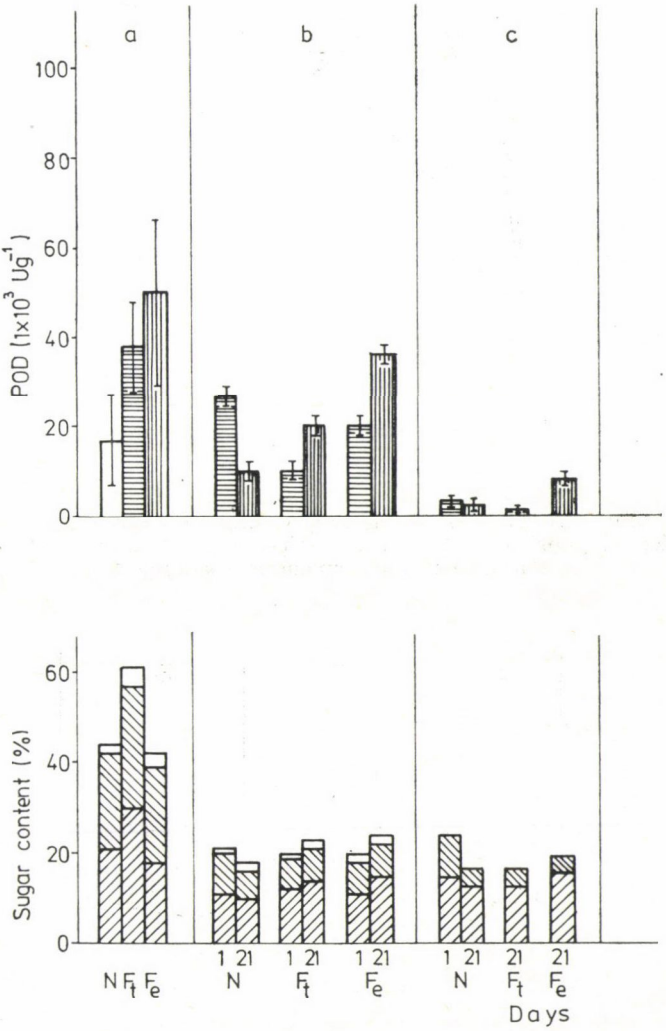


Fig. 4. Effect of after-ripening, following the application of the ripening accelerator, on peroxidase (POD) activity as well as sugar content (□: sucrose; ▨: glucose; ▧: fructose) and composition of paprika in the fresh state (*a*) and after drying at room temperature (*b*) and at 80 °C (*c*). After-ripening took place on the plant: for the rest of symbols and explanations see Fig. 3

The progress of ripening was essentially characterized by a shift in the glucose–fructose ratio towards the latter. This took place mainly between the 10th and 21st day of after-ripening following Flordimex-treatment, when POD activity had already ceased to increase or even started to decrease. In the samples of all the three lots picked on the first day the ratio of glucose

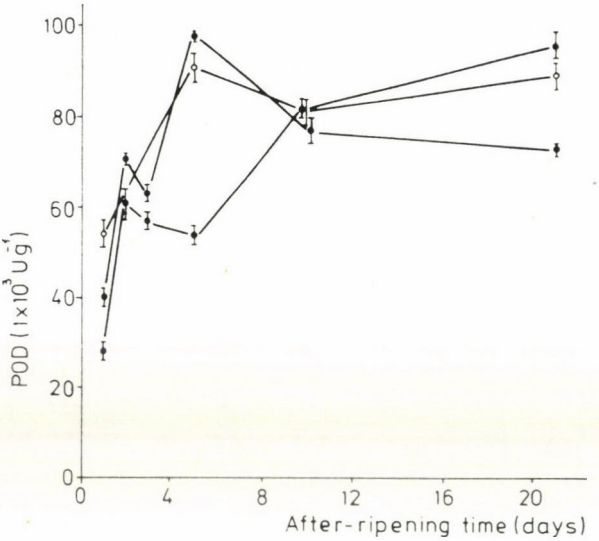


Fig. 5. Variation in peroxidase (POD) activity during after-ripening on the plant, prior to freeze-drying, of paprika treated with the ripening accelerator. (○: N, ●: $F_t$, ◑: $F_e$). For symbols and explanations see Fig. 3

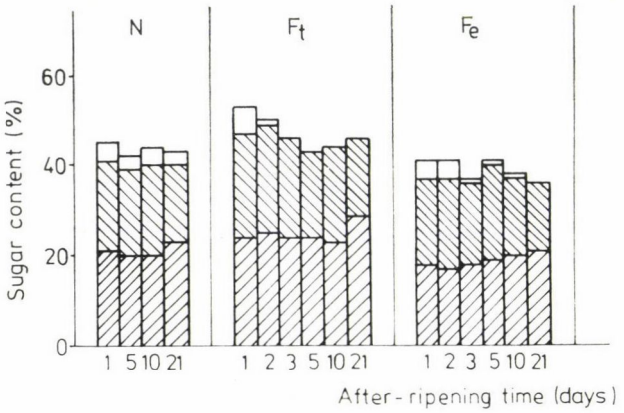


Fig. 6. Variations in sugar content (□: sucrose; ▨: glucose; ▧: fructose; w per w) and composition during after-ripening on the plant, prior to freeze-drying, of paprika treated with the ripening accelerator. For symbols and explanations see Fig. 3

to fructose was about 50 : 50, in those picked on the 21st day it was 42 : 58, 38 : 72 and 42 : 59, respectively, for the samples not treated, treated in time and treated too early.

The results of visual and instrumental colour estimation of the Flordimex-treated ground paprika-samples and the controls dried in different ways are given in Tables 2–4.

Table 2

*Comparison of the effects of the drying procedures upon visual colour rating and intrumental colour estimation data of ground red pepper samples treated with a ripening accelerator*

| Method of drying | Sample (cultivar KM 622) | | Visual rating | Instrumental estimation | | |
|---|---|---|---|---|---|---|
| | Treatment code | After-ripening (days) | | Reference sample code | $\Delta E$ | $\Delta H$ |
| *c* | $N$ | 1 | 20.7 | *b*-$N$-1 | 7.15 | 0.25 |
| | | | | *d*-$N$-1 | 21.52 | 15.31 |
| | $N$ | 21 | 18.7 | *b*-$N$-21 | 17.35 | 0.48 |
| | | | | *d*-$N$-21 | 16.88 | 5.25 |
| | $F_t$ | 21 | 22.7 | *b*-$E_t$-21 | 21.08 | 2.39 |
| | | | | *d*-$F_t$-21 | 20.98 | 7.54 |
| | $F_e$ | 21 | 15.0 | *b*-$F_e$-21 | 12.16 | 2.70 |
| | | | | *d*-$F_e$-21 | 13.22 | 7.62 |
| *b* | $N$ | 1 | 18.0 | *d*-$N$-1 | 19.51 | 16.59 |
| | $N$ | 21 | 9.0 | *d*-$N$-21 | 8.90 | 5.41 |
| | $F_t$ | 1 | 19.3 | *d*-$F_t$-1 | 23.64 | 17.31 |
| | $F_t$ | 21 | 7.0 | *d*-$F_t$-21 | 8.90 | 6.09 |
| | $F_e$ | 1 | 21.3 | *d*-$F_e$-1 | 24.50 | 19.14 |
| | $F_e$ | 21 | 8.0 | *d*-$F_e$-21 | 7.16 | 5.34 |
| *d* | $N$ | 1 | 3.0 | — | — | — |
| | $N$ | 21 | 2.0 | — | — | — |
| | $F_t$ | 1 | 4.0 | — | — | — |
| | $F_t$ | 21 | 1.0 | — | — | — |
| | $F_e$ | 1 | 6.0 | — | — | — |
| | $F_e$ | 21 | 5.0 | — | — | — |

*b* = freeze-drying
*c* = drying in an oven, at 80 °C
*d* = drying at room temperature
$N$ = not treated with Flordimex
$F_t$ = treated in time with 1 dm³ Flordimex per ha
$F_e$ = treated too early with 1 dm³ Flordimex per ha

In the reference sample code the first symbol refers to the method of drying, the second to Flordimex treatment and the third to the period of after-ripening (days). For the rest of symbols see *Table 1*

Visual rating again gave preference to the samples dried at room temperature (drying method *d*). Out of these, the sample picked 21 days after Flordimex treatment performed in time was of the best colour; the samples not treated with the ripening accelerator obtained better visual scores than those treated too early. From the freeze-dried samples those picked 21 days after Flordimex treatment were of relatively better colour.

Table 3

*Comparison of the effect of the date of Flordimex-treatment on visual colour rating and instrumental colour estimation data of ground red pepper samples*

| Method of drying | Sample (cultivar KM 622) | | Visual rating | Instrumental estimation | | |
|---|---|---|---|---|---|---|
| | Treatment code | After-ripening (days) | | Reference sample code | $\Delta E$ | $\Delta H$ |
| | $N$ | 21 | 20.7 | $c$-$F_t$-21 | 3.60 | 0.37 |
| | | | | $c$-$F_e$-21 | 4.75 | 0.91 |
| $c$ | $F_t$ | 21 | 22.7 | — | — | — |
| | $F_e$ | 21 | 15.0 | — | — | — |
| | $N$ | 1 | 18.0 | $b$-$F_t$-1 | 6.01 | 1.73 |
| | | | | $b$-$F_e$-1 | 5.88 | 4.93 |
| | $F_t$ | 1 | 19.3 | — | — | — |
| $b$ | $F_e$ | 1 | 21.3 | — | — | — |
| | $N$ | 21 | 9.0 | $b$-$F_t$-21 | 3.52 | 2.76 |
| | | | | $b$-$F_e$-21 | 1.66 | 1.29 |
| | $F_t$ | 21 | 7.0 | — | — | — |
| | $F_e$ | 21 | 8.0 | — | — | — |
| | $N$ | 1 | 3.0 | $d$-$F_t$-1 | 1.80 | 0.84 |
| | | | | $d$-$F_e$-1 | 2.48 | 2.05 |
| | $F_t$ | 1 | 4.0 | — | — | — |
| $d$ | $F_e$ | 1 | 6.0 | — | — | — |
| | $N$ | 21 | 2.0 | $d$-$F_t$-21 | 3.72 | 3.43 |
| | | | | $d$-$F_e$-21 | 2.37 | 1.36 |
| | $F_t$ | 21 | 1.0 | — | — | — |
| | $F_e$ | 21 | 5.0 | — | — | — |

For symbols see *Tables 1* and *2*.

Total and red pigment contents of the Flordimex-treated and control samples dried in different ways are given in Fig. 7.

The results of visual colour rating and pigment content are not entirely in agreement. The importance of after-ripening for pigment formation is clearly visible from the Figure. With samples dried at room temperature or by freeze-drying the application of the ripening accelerator seemed to have

a retarding effect on pigment formation. In both freeze-dried samples and those dried at room temperature pigment content was higher if the pods were after-ripened on the plant. The samples of the former group picked 21 days after the application of Flordimex yielded, irrespectively of the state of ripeness at the time of treatment, higher total pigment contents than the control sample, however, with a lower percentage of red components.

Table 4

*Comparison of the effects of after-ripening, following Flordimex treatment at different stages of ripening, on visual colour rating and instrumental colour estimation of freeze-dried ground red pepper samples*

| Sample (cultivar KM 622) | | Visual rating | Instrumental estimation | | |
|---|---|---|---|---|---|
| Treatment | After-ripening (days) | | Reference sample code[a] | $\Delta E$ | $\Delta H$ |
| $N$ | 1 | 18.0 | $N$-21 | 12.93 | 10.87 |
| $N$ | 5 | 15.7 | $N$-21 | 18.17 | 17.05 |
| $N$ | 10 | 20.7 | $N$-21 | 16.95 | 11.09 |
| $N$ | 21 | 9.0 | — | — | — |
| $F_t$ | 1 | 19.3 | $F_t$-21 | 19.65 | 15.17 |
| $F_t$ | 2 | 15.3 | $F_t$-21 | 13.45 | 11.11 |
| $F_t$ | 3 | 13.7 | $F_t$-21 | 11.45 | 10.48 |
| $F_t$ | 5 | 10.0 | $F_t$-21 | 6.69 | 6.03 |
| $F_t$ | 10 | 11.7 | $F_t$-21 | 12.09 | 10.52 |
| $F_t$ | 21 | 7.0 | — | — | — |
| $F_e$ | 1 | 21.3 | $F_e$-21 | 19.33 | 16.30 |
| $F_e$ | 2 | 25.3 | $F_e$-21 | 23.55 | 18.82 |
| $F_e$ | 3 | 24.3 | $F_e$-21 | 25.17 | 19.12 |
| $F_e$ | 5 | 22.3 | $F_e$-21 | 20.67 | 15.14 |
| $F_e$ | 10 | 11.3 | $F_e$-21 | 8.15 | 7.15 |
| $F_e$ | 21 | 8.0 | — | — | — |

[a] The symbols $N$, $F_t$ and $F_e$ stand for: non-treated with Flordimex, treated in time and treated too early, resp; 21 stands for the number of days from Flordimex-treatment till picking. For the rest of symbols see *Tables 1* and *2*.

On the whole, as to the accumulation of pigments, freeze-drying cannot compete with drying at ambient temperature, even if the pods have been after-ripened for 21 days before picking. The application of the ripening accelerator cannot remedy this either. With the samples dried at elevated temperature the ripening accelerator, when applied in time, showed a beneficial effect on pigment content.

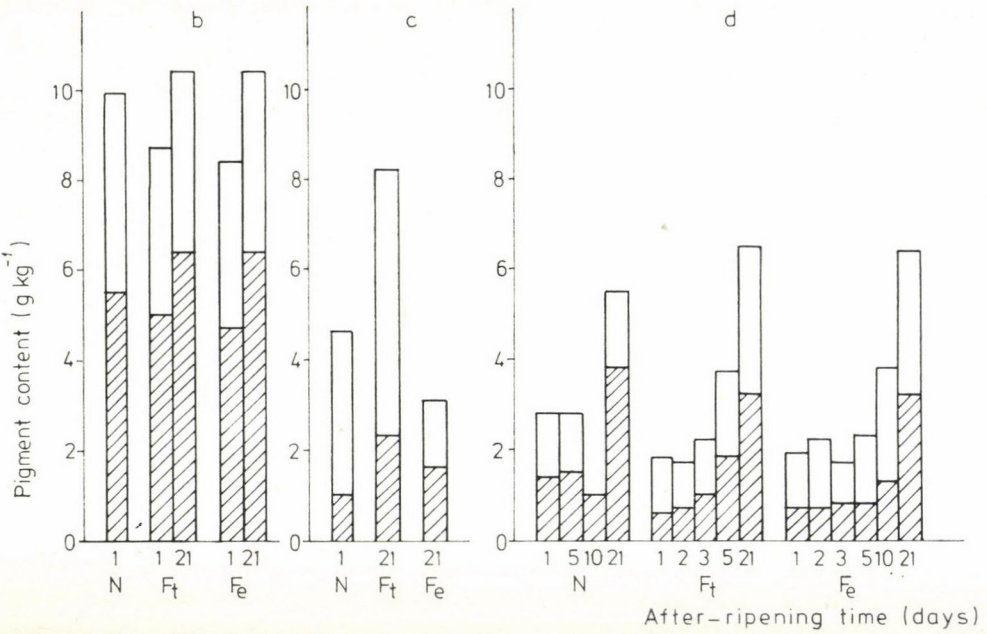


Fig. 7. Total pigment content and concentration of red components during after-ripening on the plant, prior to drying by different methods, of paprika treated with the ripening accelerator. *b*: samples dried at room temperature, *c*: samples dried at 80 °C, *d*: freeze-dried samples. Total histogram height stands for total pigment content, the shaded parts for red components

## 3. Conclusions

### *3.1. Comparison of cultivars and drying methods*

From the three cultivars analyzed in the fresh state in 1980, Sz 20 had the highest POD activity, the lowest sugar content and the best colour, KCsG 631 the lowest POD activity, the highest sugar content and the worst colour (when dried at room temperature) (Figs. 1–2, and Table 1). In a study carried out with the fresh samples of 10 paprika cultivars grown in another major paprika-growing region of the country (Szeged), good colouration was found to be accompanied by higher POD activities and low or medium sugar content, and vice versa (POLACSEK-RÁCZ & KAMPIS, 1982).

The findings concerning the colour of the samples of the three cultivars as dried at room temperature, are consistent with data published on their pigment content (KAPELLER, 1980). This has been reported to amount to 9–10.5 g kg$^{-1}$ in the cultivar Sz 20, 9–12 g kg$^{-1}$ in KM 622 and to but 7–9 g kg$^{-1}$ in KCsG 631.

The method of drying had a marked effect on all the characteristics studied. Trends of this effect were similar for the samples of all the cultivars. The decrease in POD activity (Fig. 1) during drying at room temperature to about half the value found in the fresh sample, seems to be a normal phenomenon of after-ripening (cf. Fig. 4) following picking. As in all the cases the samples dried at room temperature were of the best colour, it can be said that the residual POD activity in dry ground red pepper did not exhibit any carotene bleaching activity. In the course of after-ripening occurring during room-temperature drying, the formation of sucrose could be observed in the samples of all the cultivars.

In the samples dried at 80 °C, the drop in POD activity to a negligible value showed this treatment to be too drastic (Fig. 1). This was indicated also by the loss of glucose content and, consequently, by a considerable shift in the glucose–fructose ratio, from 35 : 65 (Sz 20), 31 : 69 (KCsG 631) and 32 : 68 (KM 622) as found in the fresh samples, to 17 : 83, 23 : 77 and 19 : 81, respectively (Fig. 2).

The high enzyme activities and sugar contents of the freeze-dried samples were due to the fact that this type of drying started with a freezing step to −40 °C and thus stopped biochemical changes immediately on picking. The analysis of "fresh" samples was carried out some days later during which after-ripening and a decrease in these parameters might have occurred. Thus, the freeze-dried samples reflected a less ripe state of the pods. This can be illustrated best by the glucose–fructose ratio in which the glucose value was somewhat more elevated than in the samples analysed in the fresh state. In the same order of cultivars as above, this ratio was, for the freeze-dried samples, 38 : 62, 39 : 61 and 35 : 65. It shall be noted that freeze-dried paprika always was of inferior colour than samples dried at room temperature. Moreover, on longer storage, freeze-dried samples often incurred heavy losses of pigments (Polacsek-Rácz et al., 1981a). This might be related to elevated POD activity, i.e. to the presence of isoenzymes capable to destroy carotenoids as was observed with unblanched quick-frozen diced carrots (Schaller & Vámos-Vigyázó, 1980). It might be assumed that during after-ripening these isoenzymes lose their activity. This is a point of great practical importance that would merit more close investigation.

### *3.2. Effects of the ripening accelerator and after-ripening before picking*

In view of the climatic conditions prevailing in Hungary, the use of ripening accelerators in paprika cultivation might be advantageous. However, an adverse effect of such treatments on pigment content and especially on the ratio of red and yellow pigments has been reported (Lendvai et al., 1981). Our own experience showed colour stability to be greatly reduced by Flordimex

treatment (POLACSEK-RÁCZ & KAMPIS, 1982), in particular, if followed by freeze-drying (POLACSEK-RÁCZ et al., 1981a). One of the difficulties of applying ripening accelerators consists in the fact that efficient treatment can be carried out only after about 80% of the pods have turned red, and even then an after-ripening period of about three weeks is required before picking to obtain a product of satisfactory quality. Consequently, as mentioned before, in the model experiments presented here the crops grown in the Flordimex-treated parcels and in the control parcel were, at the time of spraying, at different stages of ripening. This has to be borne in mind when evaluating the results.

The data concerning the variations in total sugar and sucrose contents, during chlorophyll degradation and the beginning of carotenoid accumulation in ripening paprika mesocarp, are in excellent agreement with the findings of SOUTY and ANDRÉ (1965) as well as with the theory of BENEDEK (1972). According to this author, carotenoid synthesis occurs at the expense of sugars, especially of sucrose, via organic acids, part of which serve also as source of respiration. This might account for a) the decrease in, or sometimes even disappearance of, sucrose in ripe or after-ripened samples (cf. Fig. 6) during the period of maximum pigment accumulation and b) the reappearance of this sugar in samples dried at room temperature (Fig. 2) in which carotenoid synthesis has already ceased but moisture still makes possible the occurrence of biological processes. No data were found in the literature on the order of monosaccharide accumulation in paprika mesocarp during ripening.

The increase in POD activity and in sugar content during 21-day after-ripening, prior to harvest, of Flordimex-treated paprika (Fig. 4b) indicates fundamental changes in the metabolism of the plant that could not be overcome during the slow loss of moisture on drying at room temperature, and that might account for unsatisfactory pigment formation and stability. The effect of Flordimex-treatment and subsequent after-ripening of pods on the plant can be best seen with the freeze-dried samples (Figs. 5 and 6). However, the opposite variation in POD activity and sugar content which is in disagreement with the findings obtained with samples dried at room temperature cannot be interpreted, for the time being.

The results of this experiment corroborate those of LENDVAI and co-workers (1981) as to the effect of Flordimex on pigment formation. They bear out also the findings of HARKAY-VINKLER (1974) according to which after-ripening is less advantageous on the plant than after picking. The most important conclusions to be drawn are that

— the application of the ripening accelerator cannot replace normal after-ripening and

— freeze-drying, although a mild procedure, cannot yield a product of satisfactory quality, if practised right after picking, because it blocks biological processes at a too early stage.

*

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# RELATIONSHIP BETWEEN PIGMENT CONTENT, PEROXIDASE ACTIVITY AND SUGAR COMPOSITION OF RED PEPPER *(CAPSICUM ANNUUM* L.)

## II. CHANGES OCCURRING DURING THE INDUSTRIAL DRYING PROCESS

LILLY VÁMOS-VIGYÁZÓ[a], MÁRIA POLACSEK-RÁCZ[a], ANNA KAMPIS[a], MAGDA P. PAULI[b] and GY. HORVÁTH[b]

[a] Central Food Research Institute, H-1022, Budapest, Herman Ottó út 15. Hungary
[b] County Station for Animal Hygiene and Food Control, H-6001, Kecskemét, Széchenyi krt. 29. Hungary

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Analysis of samples taken from different points of the band-driers applied for large-scale paprika processing showed the present conditions of industrial drying to cause heat damage. This resulted in the loss of total pigment content as well as a considerable decrease in peroxidase activity and sugar concentration whereby glucose was more strongly affected than fructose. The heaviest losses occurred at moisture contents below 20%. As a result of the investigations discolouration of paprika heat-dried at an industrial scale is considered to be a non-enzymatic process caused by the loss of pigments and simultaneous formation of Maillard-reaction and/or glucose caramelization products.

**Keywords:** Red pepper, pigments, peroxidase activity, industrial drying

Results of laboratory model experiments described in Part I of the present study (VÁMOS-VIGYÁZÓ et al., 1985) suggested that unfavourable changes in colouration, losses of pigment content and decrease in pigment stability during storage of powdered red pepper might be a result of heat damage occurring during artificial drying (80 °C). In order to obtain direct evidence, samples were taken from the individual stages of the industrial drying process. Beside total and red pigment as well as moisture contents, sugar concentration and composition as well as peroxidase (POD) activity were assayed in the pericarp of the pods. In previous studies (POLACSEK-RÁCZ et al., 1981; VÁMOS-VIGYÁZÓ et al., 1985) the latter characteristics had been found suited to indicate heat damage incurred on drying at elevated temperatures. The experiments were performed simultaneously with several cultivars. One of these had been treated with an ethephon-type ripening accelerator found to bring about considerable changes in the behaviour of the characteristics studied (see Part I).

## 1. Materials and methods

### *1.1. The paprika cultivars*

Beside the cultivars Sz 20 and KM 622 used in the laboratory experiments (see Part I), the cultivars K 57-231 and K 601 were applied, the latter treated, at a dose level of 1 $dm^3$ $ha^{-1}$ with the ripening accelerator Flordimex (VEB Chemie-Kombinat, Bitterfeld, GDR).

K 57-231 is an early ripening cultivar with upright pods, fire-red in the ripe and dark-red in the after-ripened state.

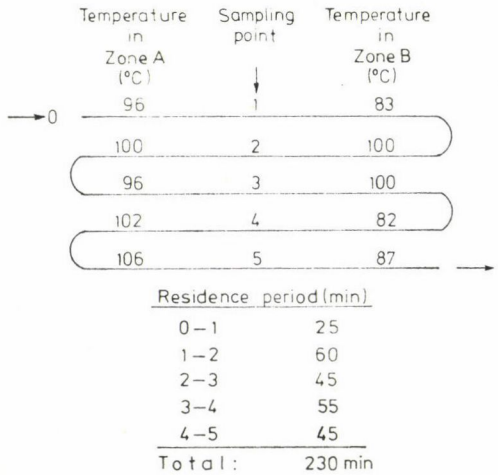
K 601 is also an early ripening cultivar, bearing a bunch of upright pods on a stalk without ramification. The pods of a given plant reach picking maturity at the same time (determinate ripening). The fully ripe pods are dark red.

Between picking and drying the pods were allowed to after-ripen at ambient temperature.

### *1.2. Analytical methods*

The determination of moisture content, glucose, fructose and sucrose concentrations as well as total and red pigment contents were carried out exactly as in Part I (VÁMOS-VIGYÁZÓ et al., 1985).

The error of POD activity determinations in fresh paprika pericarp, due to inhomogeneity of the material, could be reduced to a variation coefficient of 5% on the average, by increasing the sample size from 5 g as used earlier to 100–300 g of fresh skin. This was minced and thoroughly mixed, and a 5 g aliquot of this mass was weighed for extraction.




| Residence period (min) | |
|---|---|
| 0–1 | 25 |
| 1–2 | 60 |
| 2–3 | 45 |
| 3–4 | 55 |
| 4–5 | 45 |
| Total: | 230 min |

Fig. 1. Temperatures prevailing at the different stages of paprika drying in the Binder-type band drier, sampling points and residence periods between samplings

*1.3. Paprika sampling at different stages of the industrial scale drying process*

Samples after-ripened for two weeks following picking were taken at five points of the Binder-type (Hans Binder, Freising-Marzling, FRG) drier according to Fig. 1. The samples of higher moisture content were kept at −40 °C until the analyses, the rest (samples 4 and 5) were stored at 5–8 °C in a ventilated chamber.

## 2. Results

*2.1. Changes in peroxidase activity, pigment and moisture contents during after-ripening and drying*

POD activities, pigment and moisture contents as established in the samples of four cultivars at picking, after 12 days of after-ripening and at five different stages of drying in the Binder-type drier, are shown in Fig. 2.

At picking, POD activities in the samples of the cultivars KM 622 and K 57-231 were nearly identical, in the sample of Flordimex-treated K 601 enzyme activity was less than half that value. (This characteristic has not been determined in the fresh sample of the cultivar Sz 20, owing to sample deterioration.) Similar trends could be observed with total pigment content. At picking, the red components made up about 47% of total pigment content, their relative amounts being lowest in the sample of KM 622 and highest in that of Sz 20.

During after-ripening, POD activity decreased considerably and to different extents, in the different cultivars: in the sample of KM 622 by 32%, in that of K 57-231 by 73% and in K 601 by 45%. Simultaneously, an increase in total pigment content took place which again was different for the different cultivars: in the sample of Sz 20 it increased by about 7 rel. %, in that of K 57-231 by 57%, in those of KM 622 and K 601 by 109% and 123%, respectively. The increase in the percentage ratio of the red components was negligible in the sample of Sz 20 — in which it had been found to be highest at picking — and in the sample of Flordimex-treated K 601. In the sample of KM 622 it rose from 47.4% to 55.5% and in that of K 57-231 from 51.4% to 61.0%.

The first samples taken from the Binder-drier had higher POD activities than the after-ripened fresh samples. Similarly, total pigment content was also higher in the first samples taken from the drier, with the exception of KM 622. In this sample moisture dropped, by this time, to 30% while in the rest of the samples it still amounted to 60–70%.

In the further phases of drying the total pigment concentration decreased — more or less — in parallel with POD activity and moisture content. The red components seemed somewhat more resistant towards drying. Their relative

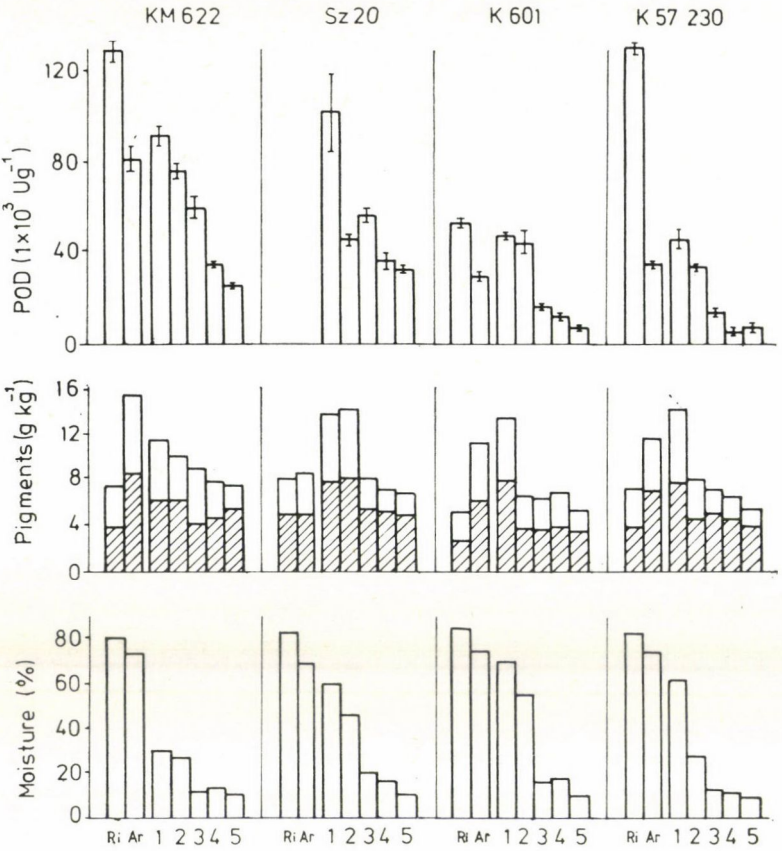



Fig. 2. Peroxidase (POD) activity, total and red pigment as well as moisture contents in paprika samples picked and analysed in the ripe state (*Ri*), after-ripened for 12 days (*Ar*) and taken from sampling points 1–5 of the Binder drier. Shaded histograms represent red pigment components, shaded + open parts total pigment content. Vertical bars represent standard deviations

amounts increased, by the end of the process, up to about 70% of total pigments, except for the Flordimex-treated sample in which they fluctuated between 56 and 60%. However, the concentration of the red components as related to solids content, incurred considerable losses during the drying process.

### *2.2. Changes in sugar content and composition*

The changes in sugar content and composition during after-ripening and drying are shown in Fig. 3.

None of the samples contained measurable amounts of sucrose.

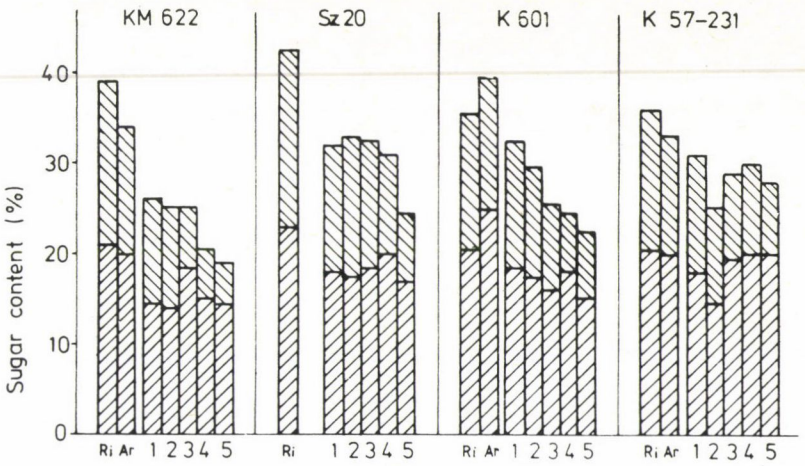



Fig. 3. Sugar content (w per w) of paprika samples picked and analyzed in the ripe state (*Ri*), after-ripened for 12 days (*Ar*) and taken from sampling points 1–5 of the Binder drier. ▨: glucose; ▧: fructose

Sugar content was highest, at picking, in the sample of Sz 20 and lowest in that of K 601. During the two weeks of after-ripening, the sugar content decreased in the samples of KM 622 and K 57-231, while it increased in that of K 601 (no assay was performed with the sample of Sz 20).

Sugar content was, at the first sampling, from the drier, lower by about 20 rel. % than in the after-ripened samples (for Sz 20, in the ripe sample). The sample of the cultivar K 57-231 showed at this stage but slight changes in sugar content.

The most dramatic changes took place at the 4th and 5th samplings. The decrease in sugar content that occurred during the drying process ranged from 10% (K 57-231) to 31% (K 601) as related to the values found at the first sampling. For the samples of Sz 20 and KM 622 the losses were very similar (22% and 25%, resp.).

The decrease in glucose content as related to total sugar content was also found to be different, in the drying process, for the samples of the different cultivars. At the first sampling, the relative glucose content was similar in all the samples and constituted 41 to 44% of total sugar content. In the sample of the cultivar KM 622 it suddenly dropped, at the third sampling, to 27%, the final value was 25%. The drop in glucose content coincided with an abrupt decrease in moisture. In the sample of the cultivar Sz 20 glucose content gradually decreased to 37% of total sugar content (4th sampling), thereafter it suddenly dropped to the final value of 30%. The most marked decrease in moisture occurred, also in this case, between the 2nd and 3rd sampling. With

the sample of the cultivar K 57-231 the most marked changes in both moisture and glucose content took place between the 1st and 2nd sampling. At the last sampling, glucose content was about 30% of total sugar content, similarly as with the sample of Sz 20. In the case of sample K 601, the greatest loss of moisture occurred between the 1st and the 2nd sampling, while the decrease in glucose content was uniform up to the 3rd sampling (from 43% to 37% of total sugar content). The final glucose concentration as related to total sugar content was 33%.

### *2.3. Correlations between the characteristics studied*

The relationship between the variations in moisture content, POD-activity and total pigment content during the drying process could be described, for the samples not treated with the ripening accelerator, by linear regression equations with two independent variables. Total pigment content was taken arbitrarily as dependent variable, without assuming any causal relationship between the variables. The equations obtained for the individual cultivars as well as the multiple determination and regression coefficients and the $F$-values indicating the closeness and the significance levels, respectively, of the correlations are given in Table 1. The values of total pigment content as measured instrumentally and calculated from the regression equations obtained for the three cultivars fit the ideal curve very closely as can be seen in Fig. 4.

For the sample treated with the ripening accelerator, no significant correlation could be established either between the three variables or, by simple linear regression analysis, between POD activity and pigment content. The correlation between total pigment content and moisture was found to be very close and highly significant ($r = 0.999$, P $> 99.9\%$).

Table 1

*Linear regression equations with two independent variables, describing the relationships between total pigment contant (TP), peroxidase activity (POD) and moisture content (M) of paprika samples at different stages of the industrial drying process*

| Cultivar | Regression equation | $R^2$ | $F$ |
|---|---|---|---|
| KM 622 | TP = 5.70 + 0.0389 POD + 0.064 M | 0.9831 | 58.09* |
| Sz 20 | TP = 6.23 − 0.0615 POD + 0.232 M | 0.9910 | 110.27** |
| K 57-231 | TP = 4.74 − 0.0509 POD + 0.187 M | 0.9754 | 39.67* |

$R^2$ = multiple determination coefficient
$F$ = result of statistical test of the correlation
* and** = correlations significant at the levels of probability of 95% and 99%, resp.

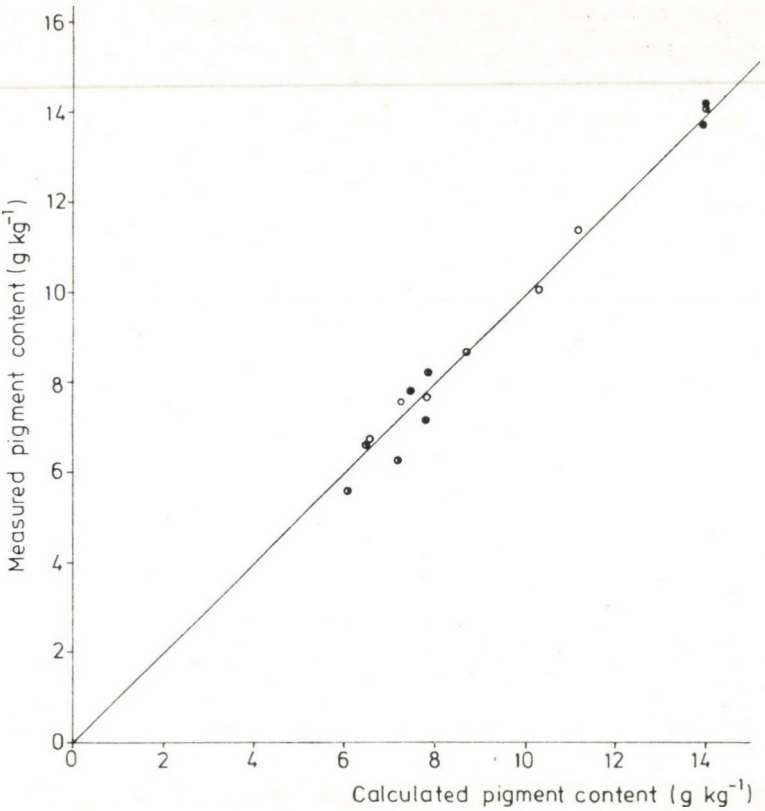



Fig. 4. Relationship between the values of total pigment content measured ($y$) and calculated ($y'$) from the relationship between this variable, moisture content and peroxidase activity in the samples taken from sampling points 1–5 of the Binder drier. Regression equation: $y = 0.000185 + 0.999\,y'$; correlation coefficients: $r^2 = 0.9849$; ($n = 15$; P > 99.9%). Cultivars: ○: KM 622; ●: Sz 20; ◑: K 57–231

Table 2

*Linear regression equations with two independent variables, describing the relationships between total pigment (TP), glucose (G) and moisture (M) contents of paprika samples at different stages of the industrial drying process*

| Cultivar | Regression equation | $R^2$ | $F$ |
|---|---|---|---|
| KM 622 | TP = 5.35 + 0.372 G + 0.0446 M | 0.9380 | 15.12$^+$ |
| Sz 20 | TP = 5.51 − 0.0746 G + 0.174 M | 0.9201 | 12.48$^+$ |
| K 57-231 | TP = 0.36 + 0.598 G + 102 M | 0.9716 | 34.19* |
| K 601 | TP = 4.99 − 0.0795 G + 0.136 M | 0.9999 | 1599.08*** |

$^+$, * and *** = correlations significant at the levels of probability of 90%, 95% and 99.9% resp.

For the rest of symbols see *Fig. 5*.

Correlations between moisture, glucose content and total pigment content (assuming the latter as dependent variable), determined at the different samplings during the drying process were found to be close for all the cultivars

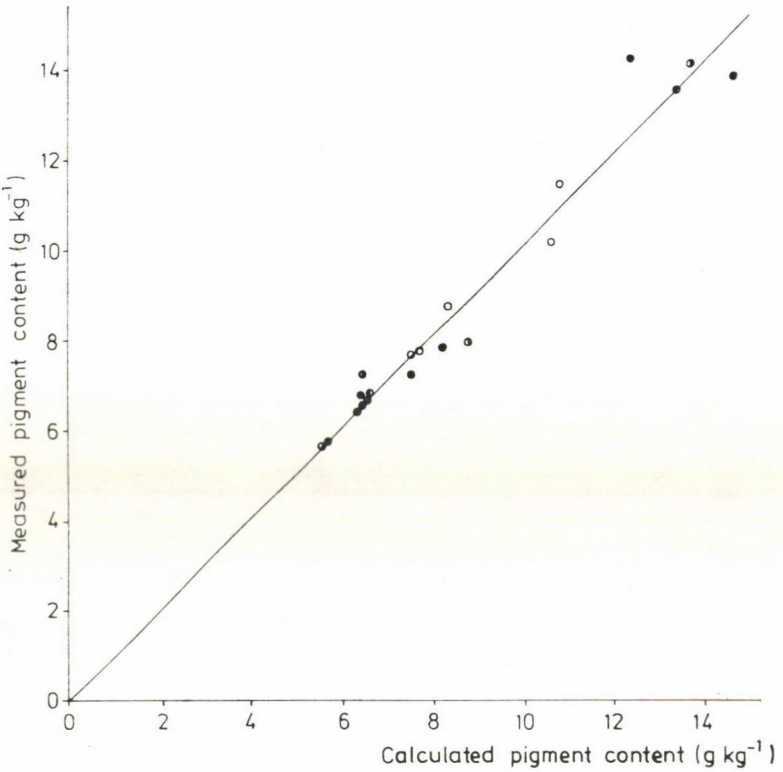



Fig. 5. Relationship between the values of total pigment content measured ($y$) and calculated ($y'$) from the relationship between this variable, moisture and glucose contents in the samples taken from sampling points 1–5 of the Binder drier. Regression equation: $y = -0.00435 + 1.000325y'$; correlation coefficients: $r^2 = 0.9616$ ($n = 20$; $P > 99.9\%$). Cultivars: ○: KM 622; ●: Sz 20; ◑: K 57–231; ◐: K 601

($R^2 > 0.95$). However, owing to the reduced degrees of freedom, some of the correlations were significant only at the 90% probability level. The regression equations, the multiple determination and correlation coefficients as well as the calculated $F$ values are given in Table 2. Fitting, to the ideal curve, of total pigment contents measured and calculated for all the four cultivars was significant at the level of probability of 99.9% (Fig. 5).

## 3. Conclusions

The absence of sucrose from the samples of the four cultivars used in the industrial drying process shows these to have been picked in the ripe state (cf. Part I). During the two weeks of after-ripening which preceded drying, the characteristics studied varied, in the samples not treated with the ripening accelerator, in the expected way: sugar (mainly glucose) content and POD activity decreased — the former but slightly, by about 10%, the latter to a greater extent, by 32 to 73% — according to cultivar. Meanwhile, pigment content increased considerably, 1.5–2-fold. In the Flordimex-treated sample pigment content increased, during after-ripening, to more than double, while sugar content rose by about 10%. This rise was due to fructose accumulation. A similar observation was made in the preceding year (Part I, Fig. 4, b). Thus, enhanced fructose formation seems to be a characteristic effect of the ripening accelerator. POD activity at picking was much lower in this sample than in the other three samples and further decreased on after-ripening. This is contrary to the results obtained in the preceding year (Part I, Fig. 4, b) and might be related to the cultivar or to differences in state of maturity, in the two years, at the time of Flordimex-spraying.

At the first sampling from the Binder drier (Fig. 2), POD activities were higher, in the samples of all the cultivars, than in the after-ripened state. The increase was significant only for the cultivars K 57-231 and K 601 and might be due to the inactivation of a heat labile inhibitor of the enzyme.

The decrease in sugar content, enzyme activity and total pigment concentration during the drying process was closely related to loss of moisture (Tables 1 and 2) which may be considered the cause of all these phenomena. POD activity of the Flordimex-treated sample was the only exception. In this sample the abrupt drop in activity followed the drastic drop in moisture with a lag of one sampling. Further experiments would be needed to decide whether the ripening accelerator had a stabilizing effect on POD. From the two sugars present, glucose proved to be much more susceptible to heat treatment. The opposite has been reported for model solution of these two monosaccharides (Kilpi & Hyvönen, 1982). This contradiction is, of course, not surprising, as the relative rates of glucose and fructose decomposition at elevated temperatures depend on a number of external factors such as water activity, pH, ionic strength, amount and composition of amino compounds, etc. (Vukov, 1983), not investigated in the present study.

From the aforesaid it can be concluded that paprika discolouration occurring during the industial drying procedure is, by no means, an enzymatic process. It may be due partly to losses in pigment content and partly to formation of Maillard reaction products or caramels from glucose. Both processes indicate heat damage which was found to be most pronounced when moisture

content dropped below 20%. Both the Maillard reaction and the decomposition of carotenoids are known to depend strongly on water activity ($a_w$). The two reactions occur at maximum rates at different water activities. For the Maillard reaction this $a_w$ is about 0.6, for oxidative carotenoid composition 0.4.

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# INFLUENCE OF HOMOGENIZATION ON THE RHEOLOGICAL BEHAVIOUR OF APRICOT PUREE

L. DURÁN and E. COSTELL

Instituto de Agroquimica y Tecnologia de Alimentos (C.S.I.C.) Jaime Roig 11., Valencia-10 Spain

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A characterization of the rheological behaviour of apricot puree, before and after homogenization, is obtained by the quantification of thixotropy by both the Weltman and the Hahn and co-workers models and by analysis of flow using both the Herschel and Bulkley mathematical model and the Michaels and Bolger structural model. Homogenization alters the product thixotropy: the initial structural flow resistance, the coefficient of thixotropic breakdown and the equilibrium shear stress were higher in homogenized purees. Homogenization did not change product pseudoplasticity ($n$) but slightly increased the consistency index ($K$), increased the yield stress ($\tau_0$) and the Bingham yield stress ($\tau_b$), and decreased the viscosity at infinite shear rate ($\eta_\infty$).

**Keywords:** Disperse systems, thixotropy, rheology, apricot puree

Particle size, shape and distribution clearly affect the rheological behaviour of disperse systems in general (SHERMAN, 1970; PARKINSON et al., 1970), and that of fruit juices and purees in particular (WITTENBERGER & NUTTING, 1958; SCHMIDT et al., 1978; SURAK et al., 1979; COSTELL & DURÁN, 1982; COSTELL et al., 1982). The influence of homogenization on the consistency of this type of products has been studied by several authors (HAND et al., 1955; CRANDALL & NELSON, 1975; BIELIG & ROUWEN, 1975; LUDNEVA & YANKOV, 1977) but a certain lack of concordance can be detected among the results from different sources. These non-coincident data and the "unexplainable" variations of consistency detected experimentally (HAND et al., 1955; CRANDALL & NELSON, 1975) could, in the opinion of the authors, be better interpreted if, instead of judging on the basis of exact measurements of viscosity, a more complete characterization of flow had been carried out.

Nowadays, sufficient instrumentation and methodology are available for the rheological characterization of this type of products (RAO 1977; RHA, 1978; DURÁN & COSTELL, 1982), allowing for a deeper and more specific analysis of the effects of processing operations. This paper follows this line or approaches and deals specifically with the effects of homogenization on the different aspects of the rheological behaviour of apricot puree and their structural interpretation.

## 1. Materials and methods

### *1.1. Materials*

Eight lots of apricot puree of the variety Canino were used. Some of their characteristics and processing conditions are shown in Table 1.

All lots were homogenized in a Manton Gaulin homogenizer, model 15M-8TA-SMD, with a Homo Valve Seat (1st stage) and Valve Seat (2nd stage), at 20.6 MPa.

### *1.2. Instrumental tests*

The methodology used for analysing the flow behaviour of apricot puree (Fig. 1) was described in a previous paper (DURÁN & COSTELL, 1982).

Rheological measurements were made in a concentric cylinder viscometer, Rheomat-15 (Contraves A.G., Switzerland) with an attached recorder. Calibration was carried out with a lubricant oil of standard viscosity. Samples were left to stand for 15 min in the viscosimeter cell before measurements. This was done at a temperature of $22.5 \pm 0.5$ °C by submerging the cell in a thermostabil bath. Cell MS/C was used.

For thixotropy quantification, torque was measured at 33.64 r.p.m. for 1 min in three sub-samples. The shear stress was calculated from the average of the three torque values registered at 6, 12, 18, 24, 30, 36, 42, 48, 54 and 60 seconds. For flow characterization, torque was measured in four sub-samples at a complete cycle of rotor speeds, first in descending order and then in ascending order to eliminate the influence of thixotropy. At each speed, a 12 s test period was employed. The shear stress corresponding to each shear rate was calculated from the average of the two torque values registered at each rotor speed.

Table 1

*Characteristics and processing conditions of apricot puree samples*

| Sample No. | Total solids (%) | | Soluble solids (°Brix) | | Pulping device | Finishing[a] holes diameter (mm) |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | | |
| 1 | 14.99 | 0.06 | 14.5 | 0.05 | Paddles | 1 |
| 2 | 14.85 | 0.02 | 14.5 | 0.07 | Paddles | 3 |
| 3 | 14.96 | 0.12 | 14.8 | 0.05 | Screw | 1 |
| 4 | 13.88 | 0.01 | 13.5 | 0.1 | Screw | 3 |
| 5 | 14.67 | 0.12 | 14.2 | 0.1 | Screw | 1 |
| 6 | 14.17 | 0.05 | 13.6 | 0.05 | Screw | 3 |
| 7 | 14.68 | 0.09 | 14.6 | 0.05 | Paddles | 0.8 |
| 8 | 14.60 | 0.01 | 14.6 | 0.05 | Paddles | 0.8 |

$\bar{x}$ = the mean value, $\pm s$ = the standard deviation of 3 measurements
[a] Paddle finisher

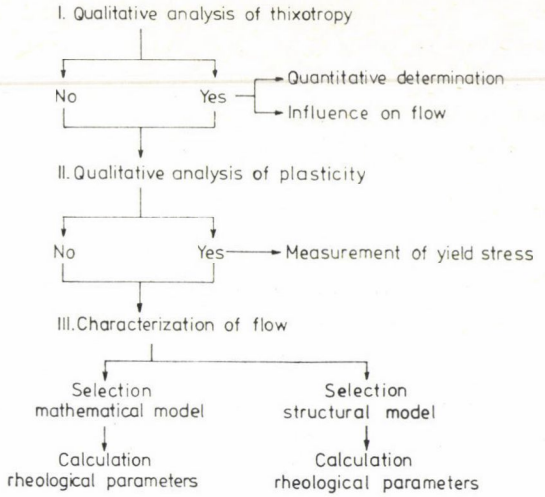


Fig. 1. Outline of general methodology for analysing rheological behaviour of fruit purees

### *1.3. Calculation of rheological parameters*

Shear stress ($\tau$) was calculated from torque, registered at each rotor speed, by using the expression of CONTRAVES (1966). Non-Newtonian shear rate ($D$) was calculated according to the KRIEGER and ELROD (1953) equation, as simplified by HAUGEN and TUNG (1976). Yield stress ($\tau_0$) was derived as the square of the intercept for the regression line between the square root of the shear stress values and the square root of non-Newtonian shear rate values (COSTELL & DURÁN, 1979).

*1.3.1. Calculation of parameters* A *and* B. Using the WELTMAN (1943) equation ($\tau = A - B \log t$), the regression line between shear stress ($\tau$) and the logarithm of time ($\log t$) was drawn. The intercept at the ordinate axis was taken as the initial structural flow resistance index ($A$) and the slope as the time coefficient of thixotropic breakdown ($B$).

*1.3.2. Calculation of parameters* $\tau_e$, p *and* a. Equilibrium shear stress ($\tau_e$) was calculated from torque registered experimentally at 33.64 r.p.m. when the torque value was constant. Using the HAHN and co-workers (1959) equation [$\log (\tau - \tau_e) = p - a\,t$], the regression line between $\log (\tau - \tau_e)$ and $t$ was drawn. The intercept at the ordinate axis was taken as the initial structural flow resistance index ($p$) and the slope as the rate of structural breakdown index ($a$).

*1.3.3. Calculation of parameters* n *and* K. Using the Herschel and Bulkley equation ($\tau = \tau_0 + KD^n$), $n$ was the slope of the line obtained by plotting $\log (\tau - \tau_0)$ versus $\log N$, where $N$ is the rotor speed (r.p.m). $K$ was the antilogarithm of the intercept at the ordinate axis when plotting $\log (\tau - \tau_0)$ versus $\log D$.

*1.3.4. Calculation of parameters* $\tau_b$ *and* $\eta_\infty$ Bingham yield stress ($\tau_b$) was the intercept of the regression line between $\tau$ and $D$ in the range 200–300 $s^{-1}$. Viscosity at infinite shear rate ($\eta_\infty$) was calculated as the square of the slope of the line obtained by plotting $\tau^{1/2}$ versus $D^{1/2}$(METZ et al., 1979). All the above mentioned calculations were carried out with an IBM 1130 computer.

## 2. Results and discussion

### *2.1. Influence of homogenization on product thixotropy*

The time dependence of the flow of apricot purees, before and after homogenization, was quantified by the parameters contained in both the WELTMAN (1943) model ($\tau = A - B \log t$) and the HAHN and co-workers (1959) model [$\log (\tau - \tau_e) = p - at$]. Both models satisfactorily explained the shear stress ($\tau$) change as a function of time ($t$), as registered experimentally at a constant shear rate ($D$). Correlation coefficients were higher than 0.95 for both models and all the samples studied. The rheological data obtained (Table 2) showed that homogenization alters the product thixotropy. The increases observed in the values of $A$ ($\Delta A > 55\%$) and of $p$ ($\Delta p > 21\%$) indicated

Table 2

*Influence of homogenization on product thixotropy*
*Rheological parameter values*

| Sample No. | Homogenization | $A$[a] (Pa) | $B$[a] (Pa · s) | $\tau$[b] (Pa) | $P$[b] (Pa) | $a$[b] ($s^{-1}$) |
|---|---|---|---|---|---|---|
| 1 | — | 28.61 | 2.07 | 23.74 | 0.146 | 0.0066 |
| | + | 57.26 | 6.23 | 35.73 | 0.219 | 0.0031 |
| 2 | — | 22.17 | 1.36 | 18.98 | 0.128 | 0.0068 |
| | + | 56.80 | 8.62 | 32.01 | 0.223 | 0.0046 |
| 3 | — | 37.69 | 4.23 | 29.22 | 0.167 | 0.0112 |
| | + | 72.57 | 9.25 | 44.10 | 0.228 | 0.0035 |
| 4 | — | 33.72 | 2.23 | 28.80 | 0.145 | 0.0078 |
| | + | 62.84 | 6.79 | 42.51 | 0.215 | 0.0042 |
| 5 | — | 41.64 | 3.70 | 33.47 | 0.167 | 0.0079 |
| | + | 69.11 | 8.64 | 47.95 | 0.211 | 0.0062 |
| 6 | — | 40.31 | 2.72 | 34.06 | 0.157 | 0.0074 |
| | + | 62.46 | 7.10 | 41.31 | 0.216 | 0.0041 |
| 7 | — | 32.65 | 2.68 | 26.74 | 0.153 | 0.0079 |
| | + | 54.03 | 5.66 | 37.59 | 0.204 | 0.0042 |
| 8 | — | 34.20 | 3.11 | 27.41 | 0.159 | 0.0082 |
| | + | 56.77 | 5.32 | 39.98 | 0.207 | 0.0037 |

[a] Weltman's equation parameters
[b] Hahn and co-workers' equation parameters

that the homogenized samples showed a higher initial structural flow resistance. The higher coefficient of thixotropic breakdown ($\Delta B = 71\%$) showed that the flow of homogenized samples exhibited higher time dependence. Lower values of parameter $a$ indicated a decrease in the rate of structural breakdown after homogenization. Finally, the increase in the equilibrium shear stress values ($\Delta\tau_e > 21\%$) showed that the homogenized product also presented higher shear resistance after the structure responsible for thixotropy was destroyed.

These results seem to confirm that in this type of products particle size reduction is not the only effect produced by homogenization. Other effects, like particle shape modification from predominantly spherical to elongated (HAND et al., 1955; ROBINSON et al., 1956) and an increase in the actual volume of the cell walls by absorption of the dispersing fluid may have a decisive influence on their rheological behaviour. The latter effect is favoured by an increase in the surface of particles as a consequence of cellular fragmentation (WITTENBERGER & NUTTING, 1958).

### 2.2. *Influence of homogenization on product flow characteristics*

After destroying the structure responsible for thixotropy in apricot puree, its flow was characterized by using both a mathematical model (Herschel and Bulkley: $\tau = \tau_0 + KD^n$) and a structural model (Michaels and Bolger: parameters $\tau_0$, $\tau_b$ and $\eta_\infty$) (DURÁN & COSTELL, 1982).

Based on the $F$ values obtained in the analyses of variance for the five rheological parameters (Table 3), it can be said that homogenization, considered as a simple effect on all samples, showed a significant ($P < 0.01\%$) influence on the values of all parameters. Homogenization produced a decrease in the average values of both the flow index ($n$) and the viscosity at infinite shear rate ($\eta_\infty$) and an increase in the following: consistency index ($K$), yield stress ($\tau_0$) and Bingham yield stress ($\tau_b$).

Table 3

*Results (F values) of analysis of variance for rheological parameters*

| | $n^a$ | $K^a$ (Pa · s$^n$) | $\tau^{ab}$ (Pa) | $\tau_b{}^b$ (Pa) | $\eta^b$ (Pa · s) |
|---|---|---|---|---|---|
| Sample | 26.56* | 22.49* | 57.69* | 356.37* | 107.83* |
| Homogenization | 19.01* | 38.04* | 280.92* | 391.99* | 90.46* |
| Interaction | 0.82 | 1.65 | 7.28* | 22.67* | 4.24* |

[a] Herschel and Bulkley parameters
[b] Michaels and Bolger parameters
* Significant ($P < 0.01\%$)

The interaction (sample × treatment) was not significant for the parameters $n$ and $K$, indicating that the effect of homogenization was similar for all samples, and confirming that these two parameters have no defined physical meaning (HOLDSWORTH, 1971); they are only quantitative measures of behavioural facts, that may originate from several causes. When considering the values of each of these two indices ($n$ and $K$) in each of the puree samples, significant differences due to treatment were not found. In fact, the small decreases in $n$ values (Fig. 2) and the small increases in $K$ values (Fig. 3)

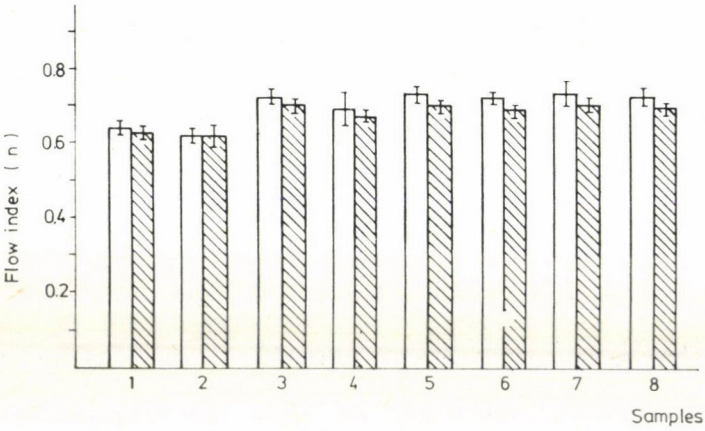


Fig. 2. Influence of homogenization on the flow index ($n$): (□) before treatment, (▨) after treatment. The vertical bars represent standard deviations

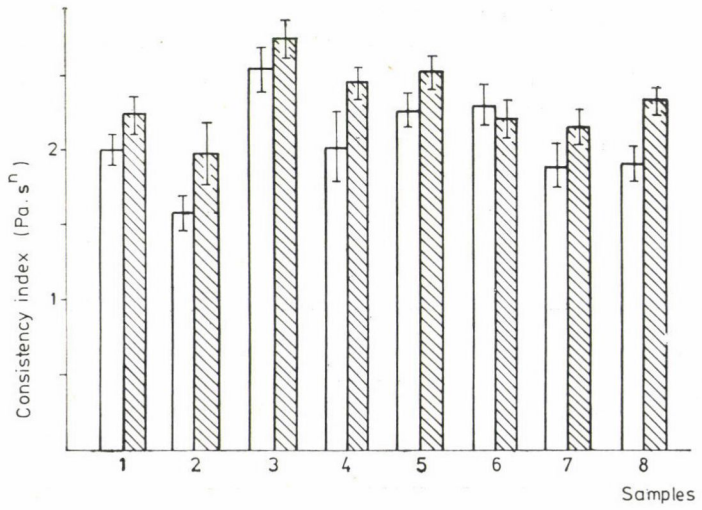


Fig. 3. Influence of homogenization on the consistency index ($K$): ( ) before treatment, (▨) after treatment

have little practical importance. These results are in agreement with those obtained by WATSON (1968) when analysing the flow properties of apricot puree prepared with fruits of different varieties and maturity indices. This author stated that homogenization did not change product pseudoplasticity ($n$ = constant), and the consistency index ($K$) increased only slightly.

The interaction (sample × treatment) was significant for the parameters $\tau_0$, $\tau_b$ and $\eta_\infty$ (Table 3). This is a clear demonstration of the structural meaning of these three parameters (DURÁN & COSTELL, 1982). The $F$ values found for these interactions (Table 3) are far lower than those for simple effects, thus indicating that, for the samples studied, the influence of such effects is much higher than that of interaction.

Theoretically, the smaller the particles, the weaker is the interparticle crosslinking responsible for the plastic properties and the lower is the flow resistance due to particle collisions (thus $\tau_0$ and $\tau_b$ values should be lower). Similarly, as higher viscosity corresponds to smaller particle size (SHERMAN, 1970), values of $\eta_\infty$ should be higher after homogenization. These theoretical relations are followed in practice when the differences in particle size are due to varietal or morphological causes (COSTELL et al., 1982), but they are not necessarily true when particle size is changed mechanically, as through homogenization.

In our experiments, an increase in $\tau_0$ and $\tau_b$ values, significant at P=0.05% probability level in all samples but one (No. 6) and a decrease in $\eta_\infty$ except for one sample (No. 2) (Figs. 4–6), were observed as clear effects of homogenization and as consequences of structural changes due to particle modifications.

These observations could be explained bearing in mind that homogenization not only produces a decrease in particle size but also causes an increase

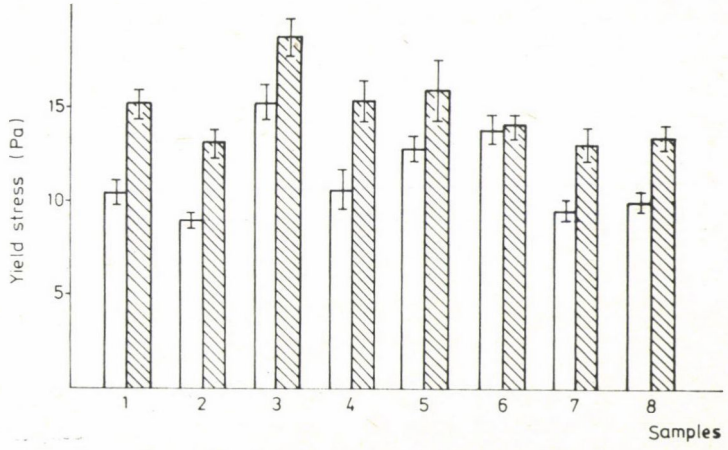


Fig. 4. Influence of homogenization on the yield stress ($\tau_0$): (□) before treatment, (▨) after treatment

in particle asymmetry and favours the absorption of water by the broken cell walls, increasing their hydrodynamic volume. The latter effects could well be responsible for the apparently abnormal variations observed in the values of $\tau_0$, $\tau_b$ and $\eta_\infty$, and are in accordance with the effects observed on studying thixotropy.

When analysing the effects of homogenization, as well as of any other treatment on the rheological behaviour of the final product, care should be

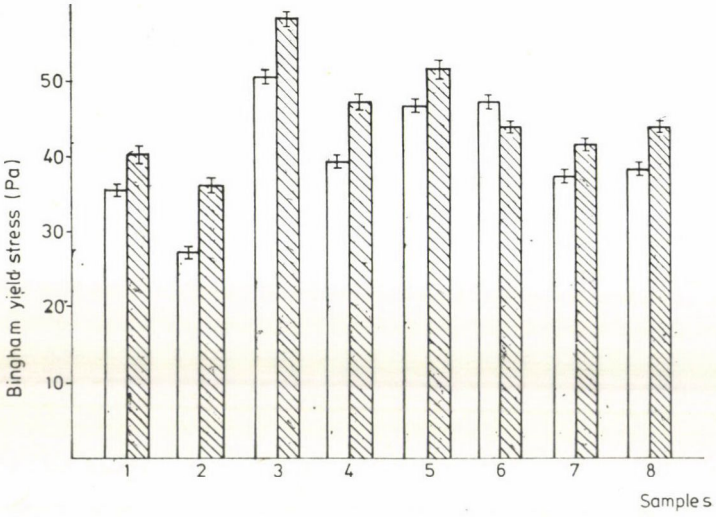


Fig. 5. Influence of homogenization on the Bingham yield stress ($\tau_0$): (□) before treatment (▨) after treatment

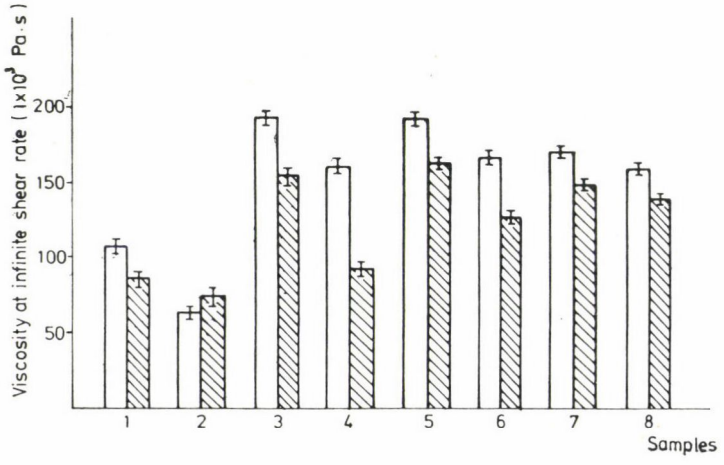


Fig. 6. Influence of homogenization on the viscosity at infinite shear rate ($\eta_\infty$): (□) before treatment, (▨) after treatment

taken in considering the real physical meaning of the chosen rheological parameter or parameters. Besides the results obtained in this paper, data from other authors illustrate this point: for example, some authors reported an increase in the consistency of tomato juice and paste, produced by homogenization (LUH et al., 1954; SMIT & NORTJE, 1958; HAND et al., 1955), while others found a decrease in the dynamic viscosity of peach puree after homogenizing (LUDNEVA & YANKOV, 1977). This apparent contradiction could well be explained by considering that, in the first case, consistency, measured in a Bostwick consistometer, depends mainly on the yield stress value ($\tau_0$) while, in the second case, the measurement of dynamic viscosity is more closely related to the value of viscosity at infinite shear rate ($\eta_\infty$).

## List of symbols

$\tau$ = shear stress (Pa)
$D$ = non-Newtonian shear rate ($s^{-1}$)
$A$ = initial structural flow resistance index (Pa)
$B$ = time coefficient of thixotropic breakdown (Pa·s
$t$ = time (s)
$\tau_e$ = equilibrium shear stress (Pa)
$p$ = initial structural flow resistance index (Pa)
$a$ = rate of structural breakdown index ($s^{-1}$)
$n$ = flow behaviour index
$K$ = consistency index (Pa·$s^n$)
$\tau_0$ = yield stress (Pa)
$\tau_b$ = Bingham yield stress (Pa)
$\eta_\infty$ = viscosity at infinite shear rate (Pa·s)

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# BOOK REVIEWS

## Testing methods in food microbiology

I. Kiss (Ed.)

Akadémiai Kiadó, Budapest, 1984, 447 pages

This laboratory manual is aimed at all those employed in microbial quality control of food. It consists of 9 main chapters dealing in a very practical way with varied aspects of food microbiology, such as laboratory design and equipment, basic microbiological procedures, sampling and sample preparation, quantitative and qualitative determination of microorganisms with particular emphasis on specific microbial groups of importance in different foodstuffs. Among the topics covered are the methods of sterilization and disinfection, preparation of culture media, microscopy, cultivation techniques, description and identification of microorganisms. Special chapters deal with the microbiological examination of environmental factors, food and additives as well as of various food commodities.

The book is the product of collaboration among prominent investigators and experts in their fields. Each chapter or part of it is by a different author but editorial control has resulted in a consistent presentation. The authors have attempted, though with different degrees of success, to bring together a collection of current knowledge on methods and recommended techniques. Preference is made of simple and effective routine methods and media easy to realize in most laboratories. However, modern rapid methods of detection, enumeration and identification receives scant treatment. Readers would certainly appreciate some information on recent development and trends. The methods concerning the investigation of pathogenic and toxigenic food-borne microorganisms are thoroughly covered. All the more is lacking a warning chapter on laboratory safety. Contrary to the presentation of pathogenic microorganisms no attempt has been made, except for canned products, to suggest in a comprehensive way procedures for detection and investigation of spoilage, although it has to be admitted that all necessary methods are described individually.

In spite of these critical remarks, the manual contains much useful information. It is intended primarily for the bench-worker in the food industry, but will be of interest to a wider readership in the food field, to students, lecturers and researchers, as well. The book is well produced, attractive in appearance. Doubtless it will be of value to those interested in food microbiology and it can be recommended with confidence to those wishing to expand their skill and practice.

T. Deák

## Solar energy for refrigeration and air conditions

Proceedings of the Joint Session of Committees $E_1$ and $E_2$ of the International Institute for Refrigeration, hold in Jerusalem, March 1982
International Institute of Refrigeration, Paris, 1982, 356 pages

Committee $E_1$ (air conditioning) and $E_2$ (heat pumps and energy recovery) held a joint meeting in Jerusalem devoted to the utilization of solar energy in refrigeration. The importance of this subject increased in consequence of the world energy crisis the sun being a "renewable", "new" source of energy. The formation of dry and wet biomass by way of chemical processes, by means of chlorophyll or the utilization of vegetable oils for

the production of thermal energy or to drive diesel engines can also be considered as the utilization of solar energy.

Heat collectors and photocells are utilized by the physical methods for use of solar energy.

The session in Jerusalem accounted mainly for refrigerating equipment connected with thermal collectors, that is for absorption refrigeration equipment.

In the presence of 60 participants coming from 16 countries and 120 Israeli participants two introductory reviews were given on the following subjects:

Tabor, H. (Israel): Utilization of solar energy in refrigeration and air-conditioning,

Mattarolo, L. (Italy): Solar powered air conditioning systems: a general survey.

The sections are as follows:

*Subject matter of Section 1:* Thermodynamic properties of binary and ternary mixtures.

Two of the four papers treated the binary systems of LiBr and/or $ZnBr_2$ solution in methanol and water —LiBr solution. The third paper treated the application of Unified Quasi-Chemical (UNIFAC) models for refrigerant-absorbent combinations and the fourth the effect of the thermodynamic properties of working fluids for absorption systems.

*Subject matter of Section 2:* Performance of solar absorption systems.

The papers were: Thermochemical heat storage for solar space heating: Solar absorption cooling installation; Study on the utilization of $CaCl_2$—$NH_3$ in a solar absorption refrigeration system; Performance of solar operated dual-stage absorption refrigeration systems with R21—DMF as working fluid; Solar powered air conditioning demonstration project in Israel: system and control design optimization; Solar powered intermittent absorption cooler; Development of an absorption refrigeration unit utilizing low grade heat; Increasing the solar fraction in solar energized air conditioning; Control and analysis of solar energized air conditioning systems.

*Subject matter of Section 3:* Other solar refrigeration systems.

The papers were: Autonomous Rankine cycle solar cooling unit; How to find the optimum process for an organic-Rankine-cycle with sensible heat sources; Vapour jet-vapour compression hybrid systems for cogeneration of cold and heat using solar energy; Ice production with a jet pump machine using solar energy; Fundamental problems in photothermal solar refrigeration; Analysis of ammoniated metal salt suspensions for the use in solar refrigeration systems; Study of the intermittent charcoal-methanol cycle for the realisation of solar powered ice-maker; Study of solar-powered ice-conservators using the day-night intermittent zoolite 13X-water cycle in temperate and tropical climates; Various possibilities of applying the zoolite 13X—$H_2O$ couple to solar cooling as a function of the type of solar energy collector; Solar air conditioning and refrigeration systems utilizing zoolites; An assessment of solar cooling in the USA; R/D programme of the Commission of the European Communities in the field of solar cooling; Performance of four refrigerators with compressors and photovoltaic cell.

*Subject matter of Section 4:* Solar heat pumps.

Titles of the papers: Portion of the heat delivered by compression and absorption heat pumps originating from the surroundings; Heat pump characteristics with evaporator modifications operated with and without solar radiation; Operation of industrial solar assisted heat pump systems with conventional R12 and R22 compressors;

*Subject matter of Section 5:* Solar collectors and available energy.

Titles of papers: Black fluid intermediate temperature collector; Evacuated selective solar collectors operating in refrigeration and air conditioning systems; The use of solar ponds for air conditioning; Comparison of weather data of different time resolution as applied to a simulation of an indoor swimming pool; The availability of solar radiation in Beer-Sheva (the Negev region); Collection and processing of solar radiation data in the Etna region.

*Subject matter of Section 6:* Architectural problems.

Papers read: A parametric study for optimal design of windows; Characteristic parameters of windows; Simulation of solar office heating system with air collectors and a rock bed store; Analysis of different types of climatic architecture.

As judged by the titles of the papers a world-wide effort is made to utilize solar energy, to the maximum possible extent, for refrigeration and air conditioning. Most of the participants reading a paper came from countries of warmer climate (Israel, India, Mexico) but there were also Danish, Belgian, Dutch and German participants which shows that this subject is of interest in countries of colder climate than that of Hungary, too. Perhaps the treatment of the subject would deserve a greater effort here, too.

E. Almási

# EDITORAL

With regret it is annouced that

Professor Elemér ALMÁSI

State Prize winner, one of the founding members of *Acta Alimentaria* passed away in Budapest on 23rd April 1985. The obituary will be published in the next issue (Vol. 14, No. 3).

# ANNOUNCEMENT

CONGRESS OF THE INTERNATIONAL SOCIETY FOR RESEARCH ON CIVILIZATION DISEASES AND ENVIRONMENT

Budapest, Hungary, October 23—25, 1985

Organized by: Federation of Hungarian Medical Societies

Office for Conference Organization: Budapest, P.O. Box 32 H-1361 (Telex: 22-4204; telephone: 125—012)

Main topics of the Congress:
Chronobiology and Nutrition
Health and Nutrition
Nutrition as Risk Factor
Nutrition and Caries Prophylaxis
Nutrition in Education
Interaction between Pharmaceuticals and Diet
Round table: Dietary Fibre in Nutrition

## Editorial Note

The Editorial Office informs the Reader that the book

*Microbial Associations and Interactions in Food,*

Proceedings of the 12th International IUMS—ISFMH Symposium, Budapest, Hungary, 12—15 July 1983. Eds: I. Kiss, T. Deák, K. Incze
has been published jointly by D. Reidel, Dordrecht—Boston and Akadémiai Kiadó, Budapest in 1984. The volume contains all the papers presented at the Symposium.

The Abstracts of the papers were published in Acta Alimentaria, Vol. 13, pp. 233—239.

# RECENTLY ACCEPTED PAPERS

Viability of food poisoning microorganisms in Egyptian salads
ZAKIA A. HELMY, AFAF ABD-EL-BAKEY & DAW, Z. Y.

Separation of, and investigation into the properties of trypsin and chymotrypsin from an ovine + caprine pancreatic enzyme preparation. — Part I. and II.
ZHIGZHIDDORZHIN, A., BOROSS, L. & VÁMOS-VIGYÁZÓ, L.

Effects of some additives and processes on the characteristics of agglomerated and granulated spray-dried Roselle powder
EL TINAY, A. H. & ISMAIL, I. A.

# NOTICE TO CONTRIBUTORS

*General.* Manuscripts in English or Hungarian should be typed double-spaced on one side of the sheet and should not exceed 20 pages.

*Title.* The title should be concise and informative. It must be followed by the authors' names and the address(es) of the institute(s) where the work was carried out.

*Summary.* The article should be preceded by a summary (not exceeding 150 words) giving a self-explanatory compendium of the essence of the paper. At the end of the summary some 4 keywords should be put in alphabetical order.

*Text.* The article should be divided into the following parts: *Introduction* beginning on a new page without a title containing a survey of related literature and the objectives of the work, followed by *Materials and methods; Results; Conclusions.*

*Symbols.* Units, symbols and abbreviations should be used according to SI (System International) rules. Unusual abbreviations must be explained in the text or in an appendix.

*Figures and tables.* Illustrations must be cited and numbered in the order they appear in the text. All line drawings should be submitted as clear, glossy, black and white originals or photographs of good quality. The author's name and the title of the paper together with the serial number of the figure should be written on the back of each figure. Figures and Tables, each bearing a title, should be self-explanatory and numbered consecutively. The number of measurements (n), their mean values ($\bar{x}$) and standard deviations ($\pm s$) should be indicated. To prove the objectivity of conclusions drawn from the results mathematical statistical methods must be used. Quantitative evaluation of data is indispensable.

*References.* The reference list (Literature) should be in alphabetical order as follows:

*Periodicals:* Names and initials of all the authors; year of publication in parentheses; colon; title of the paper; title of the periodical; inclusive page numbers.

*Books:* Names and initials of all the authors; the year of publication in parentheses; colon; title of the book; publishing firm, place of publication; inclusive page numbers.

Detailed instructions for publishing in Acta Alimentaria are available from the Editorial Office.

Authors will receive one set of proofs which must be corrected and returned at the earliest convenience to the Editorial Office. In this phase major alterations of the text cannot be accepted.

*Acta Alimentaria* is surveyed by Current Contents.

# ACTA AGRONOMICA

## ACADEMIAE SCIENTIARUM HUNGARICAE

EDITED BY

S. RAJKI and GY. PÁL

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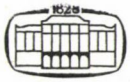

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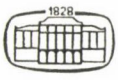
VOLUME 14

1985



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BUDAPEST



D. REIDEL PUBLISHING COMPANY
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*Acta Alimentaria, Vol. 14 (3), pp. 213—229 (1985)*

# BIOCHEMICAL STUDIES ON IRRADIATED ONIONS, POTATOES AND MUSHROOMS

B. MATKOVICS

Biological Isotope Laboratory, József Attila University of Szeged, H-6701 Szeged, P.O.Box 539. Hungary

(Received: 30 March 1983; revision received: 1 March 1984; accepted: 5 March 1984)

The supply of mankind with food of good quality and appropriate nutritive value is an important problem. Attention has long been focussed on the preservation of basic foodstuffs by ionizing radiation. Radiation treatment is performed under aerobic conditions, and it is well known that oxygen radicals are formed in aqueous medium in response to ionizing rays. Accordingly, it appeared important to study how the activities of the antioxidant enzymes: superoxide dismutase (SOD, EC 1.15.1.1), peroxidase (P-ase, EC 1.11.1.7) and catalase (C-ase, EC 1.11.1.6), and the quantitative values of some other important components of irradiated foodstuffs vary at different times subsequent to irradiation. In all cases, the control was the basic foodstuff stored under identical conditions but not irradiated.

It may be stated in general that the activities of the examined antioxidant enzymes and the value of lipid peroxidation (LP) vary from species to species, but also depend on the variety within the species.

For instance, in one onion variety the antioxidant enzyme activity changes develop more quickly after irradiation, and remain for a longer time, than in another variety.

Dynamic changes of the smaller molecules, lasting for various periods of time, can also be well observed following irradiation. Similarly as previously, these indicate that radiation treatment changes the physiological processes of the examined basic foodstuffs and give rise to new equilibria in these processes.

**Keywords:** irradiation of foodstuffs, onions, potatoes, mushrooms

The literature survey given here, together with our own results, will be restricted to oxidative changes following gamma-irradiation. As regards the antioxidant systems, our measurements were extended over the antioxidant enzymes (see above). Information on the oxidative damage is provided by lipid peroxidation measurements, and indirectly the other examined components, too, relate to the redox changes subsequent to irradiation.

To take a simple example, we know that if aqueous solutions are subjected to radiolysis under aerobic conditions, there is a considerable primary or secondary formation of oxygen radicals (OH, $O_2^-$), $e_{aq}^-$ (which may similarly lead to the formation of oxygen radicals) and $H_2O_2$ (SCHWARZ, 1981). Naturally, the foodstuffs we have examined can not be regarded as such simple systems, but the results described in the paper of Schwarz generally refer to biological systems, too.

Of the secondary reactions, mention should be made of the Haber-Weiss and Fenton reactions: the biological significance of these is still disputed in

many respects, but it is highly probable that they can be conceived as the source of further $OH^{\cdot}$ radicals in both animal and plant tissues (HALLIWELL, 1978).

A list follows of the reactions catalyzed by the enzymes of interest, and of the two secondary reactions mentioned above:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \quad (1)$$

$O_2^-$ = superoxide anion radical

$$H_2O_2 + AH_2 \xrightarrow{\text{P-ase}} 2H_2O + A^{\cdot} + (^1O_2)\ (?) \quad (2)$$

$^1O_2$ = singlet oxygen
$AH_2$ = proton donor
$A^{\cdot}$ = proton donor residue

$$H_2O_2 + H_2O_2 \xrightarrow{\text{C-ase}} 2H_2O + O_2 + (^1O_2)\ (?) \quad (3)$$

Haber-Weiss reaction:

$$H_2O_2 + O_2^- \rightarrow OH^+ + O_2 + OH^{\cdot} \quad (4)$$

Fenton reaction:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + H_2O \quad (5)$$

The basic foodstuffs examined were onions, potatoes and mushrooms.

## 1. Materials and methods

The labor scale $^{60}Co$ apparatus situated in the Central Food Research Institute (Budapest) was used as radiation source, its dose was 5 kGy $h^{-1}$. The samples were received either on the day following irradiation day, or later stored under the same conditions as the control materials, and sampled for analysis at the times given in the Figures.

Two onion varieties, Makó and Alsógöd, were examined. The onions were stored either in a cellar or in open air and in the winter they were covered with straw. In all cases the plant parts analysed comprised a sample excised from the second leaf (L), shoot (S), and the root stem (T).

Similarly, two potato varieties, Somogy gyöngye and Désirée II, were examined. The potatoes were stored in a cellar during the study. Here the enzyme activities and other parameters were investigated in homogenates of the peel (P), the storage tissue (ST) and the bud (B).

One type of mushroom *(Psalliota campestris)* was also examined. The mushrooms were kept at 4 °C in a refrigerator, and analyses were made on the cap (C), the lamella (SP) and the stem (S).

Homogenization was performed either with quartz sand or in a Potter homogenizer. In general a sample of about 1 g wet weight was taken. For the SOD measurement this was homogenized in 0.1 mol $K_2HPO_4$, and for the other analyses in a 10-fold volume of physiological NaCl solution. After centrifugation of the homogenates, the supernatants were used for enzyme activity determinations and other quantitative measurements.

The following enzyme activities were determined: SOD activity was measured by the method of McCord and Fridovich (1969). This measurement is based on the observation that the epinephrine-adrenochrome transformation is inhibited by SOD in a dose-dependent manner. In this method, but generally in all the indirect enzyme activity determinations, the unit of enzyme activity is the quantity of enzyme that under the described conditions causes a 50% inhibition of the epinephrine-adrenochrome transformation. The extent of inhibition may be determined spectrophotometrically at 480 nm (Matkovics et al., 1977).

Peroxidase activity was measured by the well-proved spectrophotometric method at 470 nm, where the cosubstrate of $H_2O_2$ is guaiacol (Chance & Maehly, 1955).

Catalase activity was determined at 240 nm on the basis of the time-dependent decomposition of $H_2O_2$. It is expressed in Bergmeyer units (BU) in the Figures, 1 BU means the consumption of 1 g $H_2O_2$ in 1 min (Beers & Sizer, 1952).

Other quantitatively measured components:

— Measurements representing oxidative metabolism

= Determination of lipid peroxidation. LP gives a picture primarily of the metabolism of polyunsaturated fatty acids involving oxygen radicals. In every case we measured the total reactive oxo or dioxo compounds formed during LP, and the results being given in malondialdehyde (MDA) values, was measured photometrically at 532 nm after reaction with thiobarbituric acid. LP activities are given as mol (MDA) per g wet tissue (Placer et al., 1966).

— Quantitative determinations only indirectly related to the oxidative metabolism

= Glucose was determined at 620 nm, via its colour reaction with o-toluidine (Hyvarinen & Nikkila, 1962).

= Ascorbic acid was measured spectrophotometrically at 546 nm after reaction with 2,4-dinitrophenolhydrazine (Sós, 1974).

= Protein was measured with the Folin reagent at 675 nm, by the spectrophotometric method of Lowry and co-workers (1951).

The total pectin and the fractions soluble in water, in 0.1 *N* NaOH and in $NH_4$-oxalate were determined spectrophotometrically by a combination of the methods of Pozsár-Hajnal and Polacsek-Rácz (1975), Pozsár–Hajnal and co-workers (1977), Zitko and Rosik (1961).

The error of the enzyme activity measurements was $\pm$ 10%. The columns in the Figures give the means and the standard deviation ($\pm$S.E.) around the mean for at least 10 measurements.

In general, the onions, potatoes and mushrooms were stored under optimum conditions. In every case, efforts were made to model the standard optimum storage conditions applied commercially.

## 2. Results

Figures 1–3 compare SOD, P-ase and LP activities of the two onion varieties (Alsógöd and Makó) irradiated with 50 Gy and stored under the same conditions, and of one potato variety (Somogy gyöngye) irradiated with 80 Gy, with the corresponding activities of the same tissue homogenates from non-irradiated controls. The SOD activities are compared in Fig. 1 for both onion varieties and potato. A difference may be detected primarily in the SOD activity of the root stem of the Alsógöd onion. The SOD activity is higher in the homogenates of the leaf, shoot and root stem of the Makó onion on the 37th day following irradiation. The elevated enzyme activities of the onion root stem may be related to the fact that irradiation results also in the inhibition of root growth, and the $O_2^-$-caused induction of SOD is responsible for the SOD activity increase. In this series SOD measurements on potato are deficient, but the enhanced radiation sensitivity and reaction of the potato tissues with higher water content are striking.

Figure 2 gives the P-ase data in samples taken at the same times as in Fig. 1. The P-ase activities show significant differences at both points of time in the Alsógöd onion shoot (S) and root stem (T) and in the potato peel (P) and bud (B). The shoot and root stem display significant differences on the 37th day following irradiation in the Makó onion.

Figure 3 gives the various tissue LP activities in units of nmol malondialdehyde (MDA) per g wet tissue. The activities are higher in the later sample for the Alsógöd onion, but significantly lower on the 37th day than on the 2nd day for the Makó onion. The LP activity is significantly higher in the B homogenate of the potatoes. (This is probably connected with the inhibition of germination in the potatoes and Alsógöd onions.)

The SOD, P-ase and C-ase activities on the 1st, 6th, 20th and 27th days are compared in Figs. 4–6 (C-ase was determined only on the 6th and 20th days, see Fig. 6).

In other Figures glucose, ascorbic acid and pectin contents are compared for the onion and potato samples at four different times.

In Fig. 7 the free glucose contents of Alsógöd onions irradiated and non-irradiated are compared, stored in the open under the same conditions. Homog-

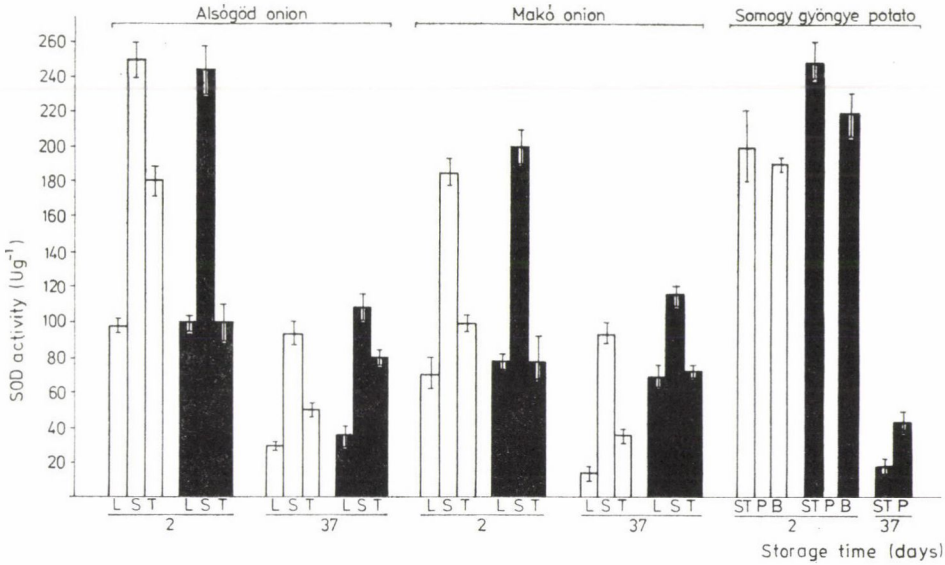


Fig. 1. SOD activities in irradiated (50, 80 Gy resp., ■) and control (□) homogenates of different onions and potatoes. Leaf (L), shoot (S) and root stem (T) of Makó and Alsógöd onions and of Somogy gyöngye potato peel (P), storage tissue (ST) and bud (B) on the 2nd and 37th days

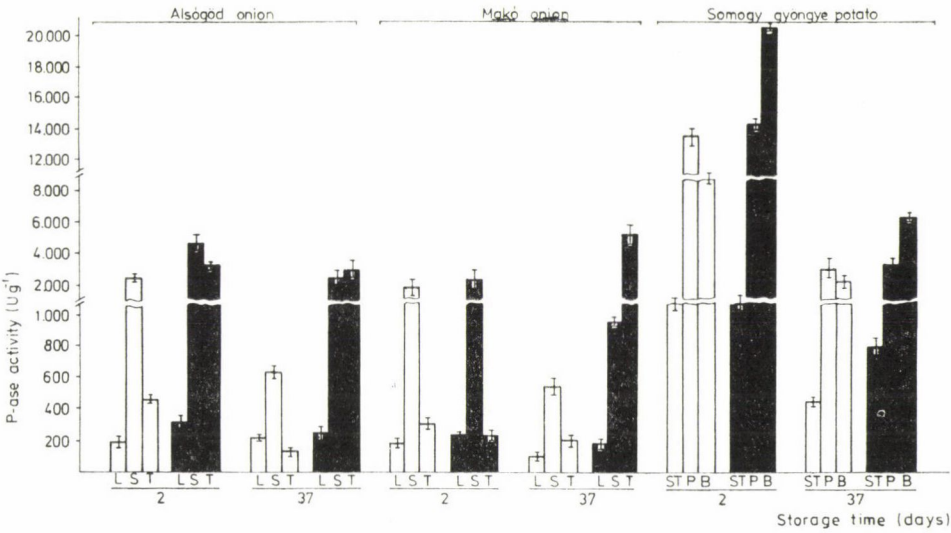


Fig. 2. P-ase activities of potatoes and onions. For further data see Fig. 1

enates of the same parts of the samples, taken on 30 October, 29 November, 4 January and 6 February were used for analysis.

In Fig. 8 the free glucose contents of control and irradiated Makó onions are compared.

With Alsógöd onion, the main difference is observed in the glucose content of the bud homogenates: that for the irradiated sample is lower than that for the control, the latter increasing as a function of time (Fig. 7). With the Makó onion, a similar difference is observed in the bud, but even more markedly in the stalk homogenates (Fig. 8).

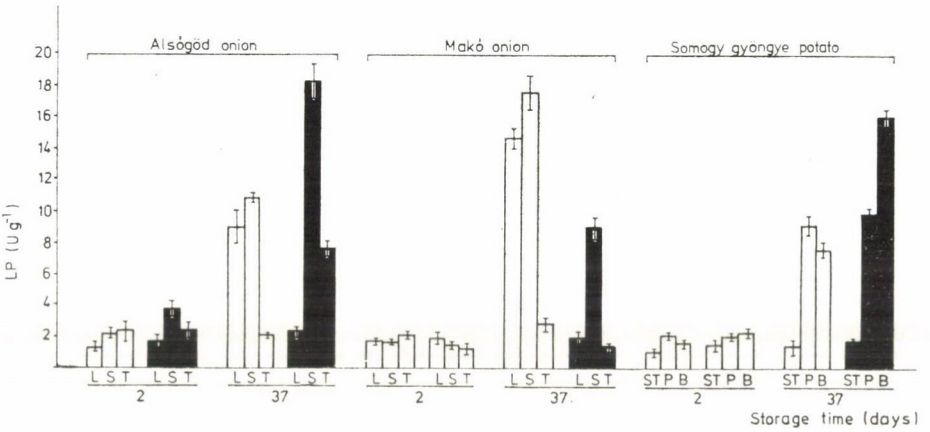


Fig. 3. LP values of irradiated (■) onions and potatoes and untreated controls (□). LP activities are expressed in units of nmol malonaldehyde per g wet tissue. Symbols as in Fig. 1

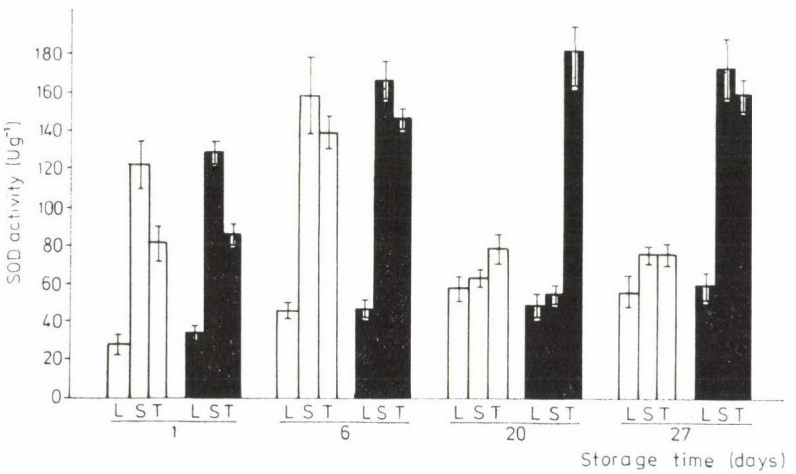


Fig. 4. SOD activities of irradiated (50 Gy; ■) and non-irradiated (□) commercial onion parts, stored under the same conditions

In Figs. 9 and 10 the ascorbic acid contents of the Alsógöd and Makó onions are compared at the above sampling times. With the Alsógöd onion, differences are found in the S and T homogenates of the two samples at the 2nd sampling time, and mainly in the S homogenates at the 3rd and 4th sampling

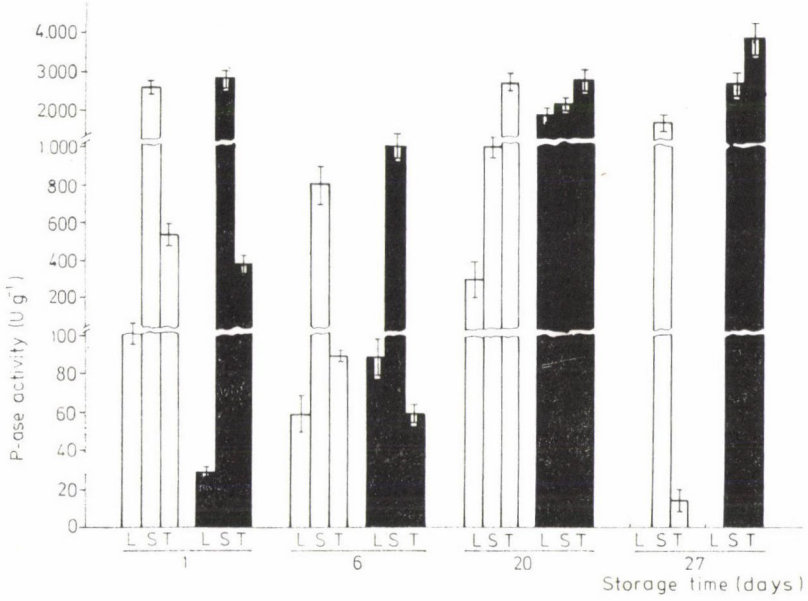


Fig. 5. P-ase activities of the same onion parts as in Fig. 4 (Storage time 1, 6, 20 and 27 days after irradiation). ■: Irradiated with 50 Gy; □: control

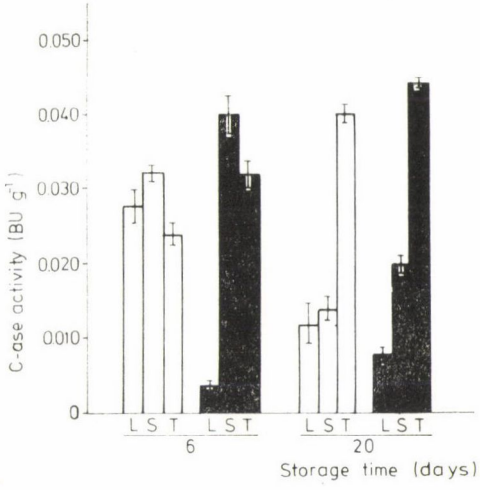


Fig. 6. C-ase activities of commercial onion samples 6 and 20 days after irradiation. BU: Bergmayer unit (1 BU: consumption of 1 g $H_2O_2$ in 1 min). ■: Irradiated with 50 Gy; □: control

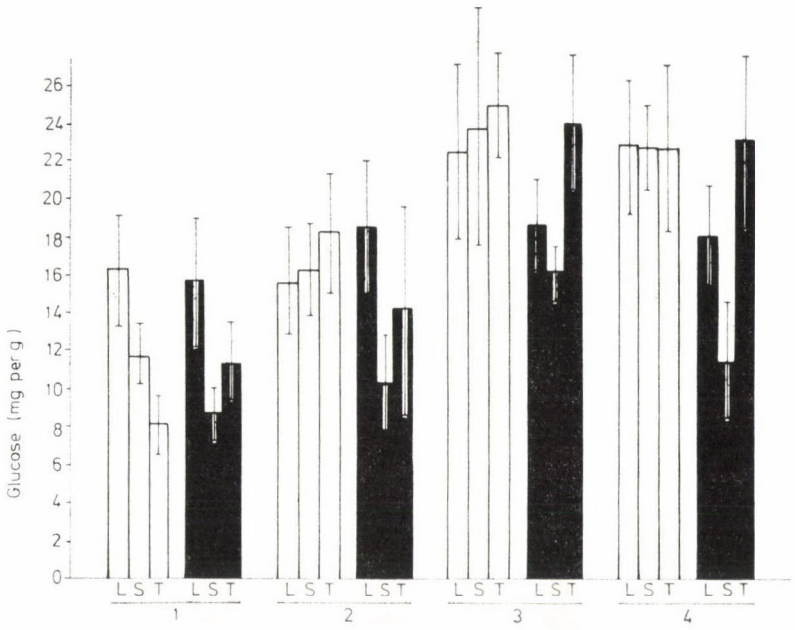


**Fig. 7.** Glucose contents of irradiated and control Alsógöd onion, in random samples taken from material stored under the same conditions. Sampling dates: 1=30 October, 2=29 November, 3=4 January and 4=6 February; ■: irradiated with 50 Gy; □: control

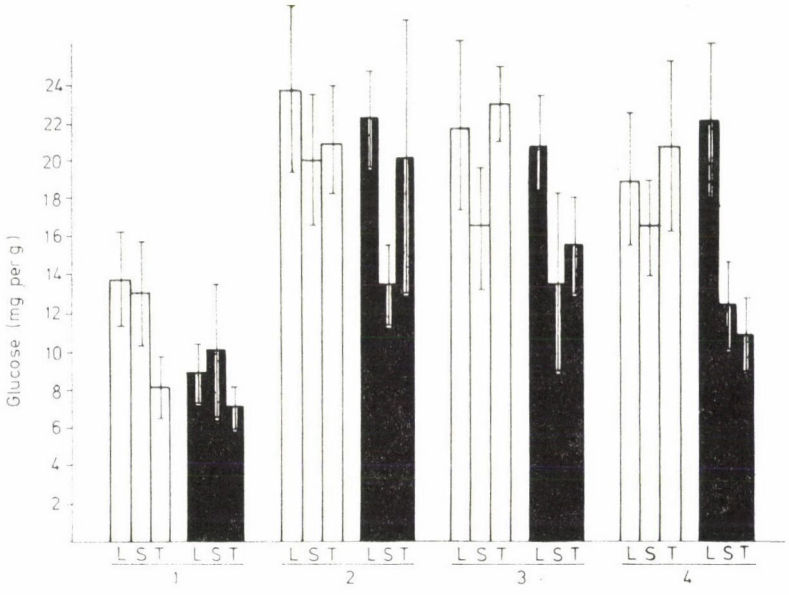


**Fig. 8.** Glucose contents of irradiated and control Makó onion parts. For details see Fig. 7

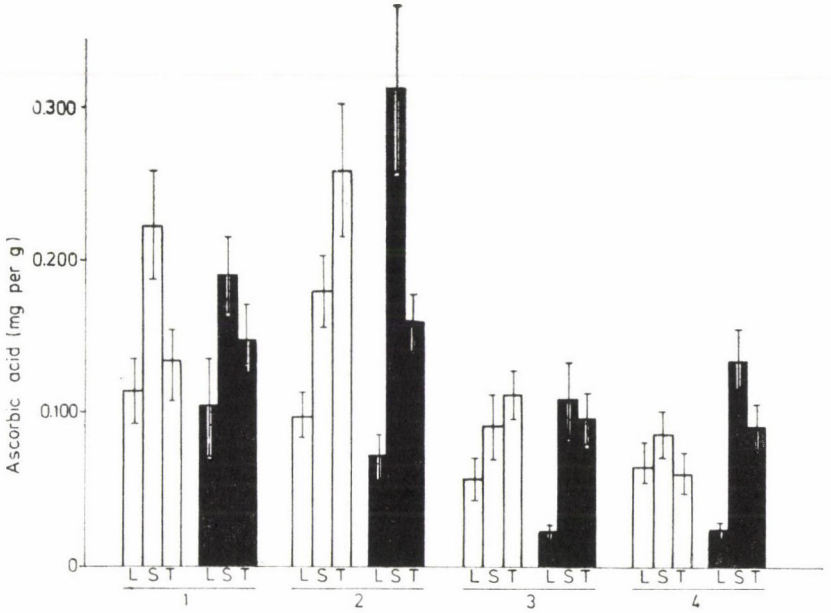


Fig. 9. Ascorbic acid contents of irradiated and control Alsógöd onion parts. For details see Fig. 7

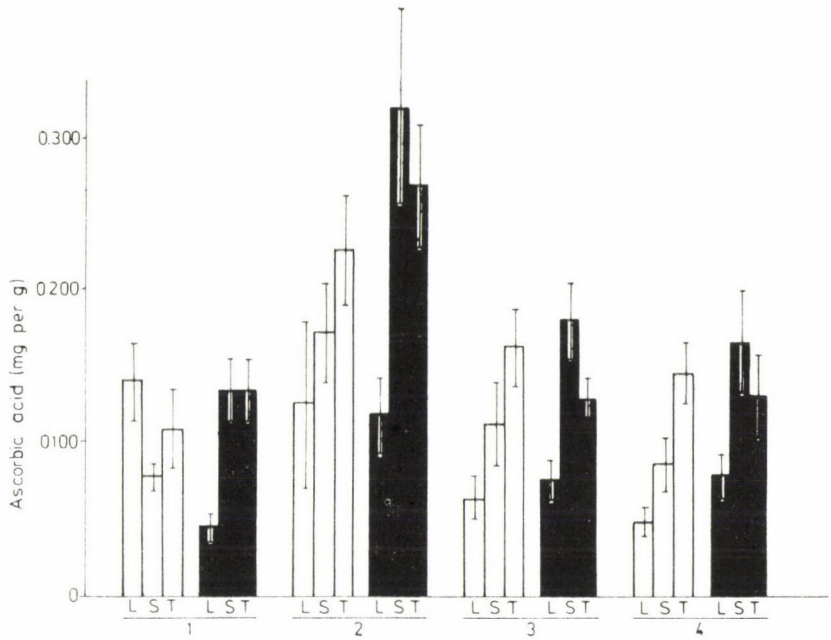


Fig. 10. Ascorbic acid contents of Makó onion parts as functions of storage time and treatment. For details see Fig. 7

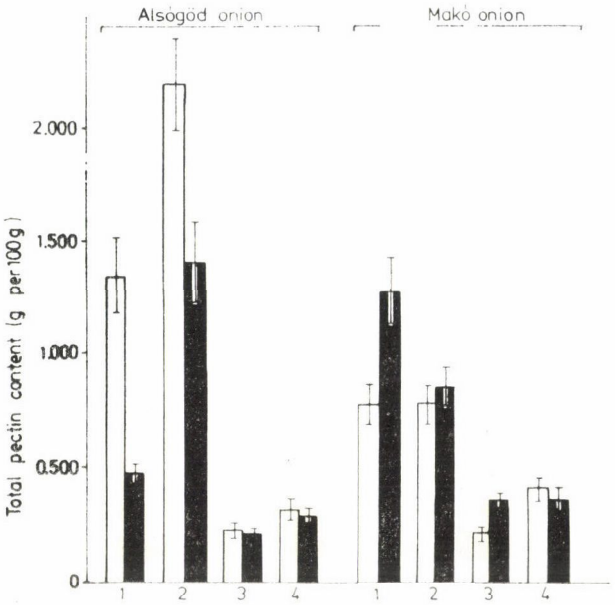


Fig. 11. Total pectin contents of irradiated and control Alsógöd and Makó onions. For details see Fig. 7

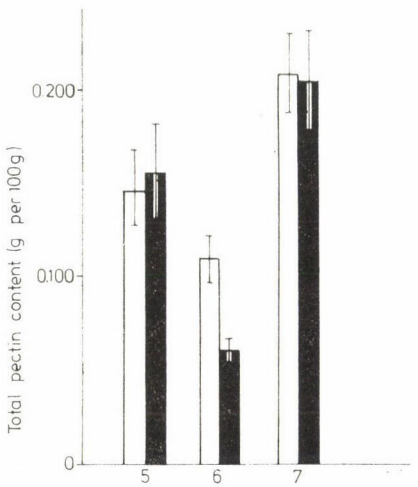


Fig. 12. Total pectin content in Desirée II potato tissues during storage at various sampling times. Control (□) and irradiated (80 Gy, ■) potatoes stored under the same conditions. Sampling dates: 5=7 March; 6=13 April; 7=14 May

times. With the Makó onion, the leaves (L) display a significant difference from the control at the 1st sampling, but in the later samples the difference diminishes.

Figure 11 presents a comparison of the total pectin contents of the two varieties of onion. This illustrates very clearly that the pectin level decreases during storage, and shows that the pectin content is variety-dependent.

In Fig. 12 the total pectin contents in storage tissue homogenates in samples taken of the potato variety on three occasions, are compared.

A brief account of the studies relating to the irradiation of mushroom *(Psalliota campestris)* is also given. The mushroom was exposed to 2.5 kGy irradiation, and in one series the P-ase, C-ase, LP, protein, glucose and ascorbic acid were determined on the 2nd, 3rd and 4th days. The results are presented in Figs. 13–18. The parts of the mushroom examined in homogenate form were the cap (C), the lamella (SP) and the stalk (S).

In Fig. 13 the P-ase activities are compared. A significant change can not be demonstrated initially, but on the 3rd day, and particularly on the 4th day the P-ase activity decreased significantly in all parts examined. More or less the same can be said for the C-ase activity of the mushroom parts (Fig. 14).

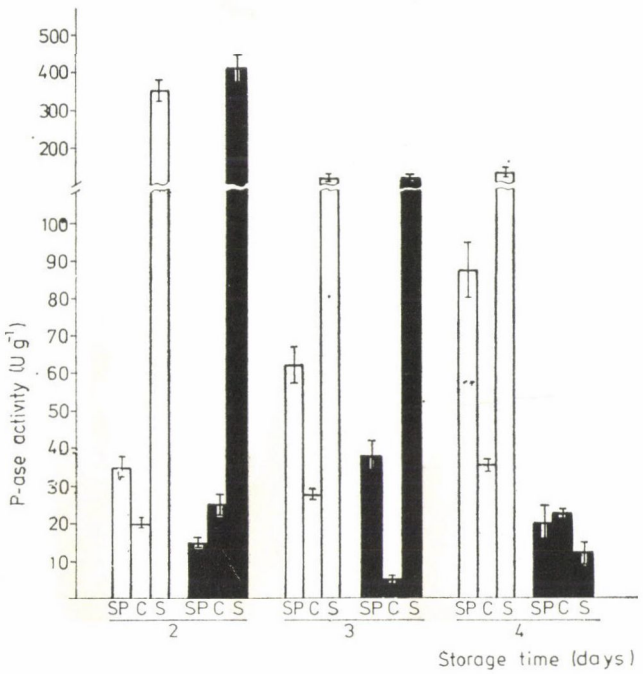


Fig. 13. P-ase contents of irradiated (▬) and control (□) mushroom parts: lamellae (SP), cap (C) and stem (S) homogenates, 2, 3 and 4 days after 2.5 kGy irradiation. Mushrooms stored at +4 °C

There are no essential differences between the irradiated and the control mushroom parts as regards LP and the amount of protein (Figs 15–16).

In Figs. 17 and 18 the quantitative changes in the glucose and ascorbic acid contents are compared. In the stalk the amount of glucose is significantly

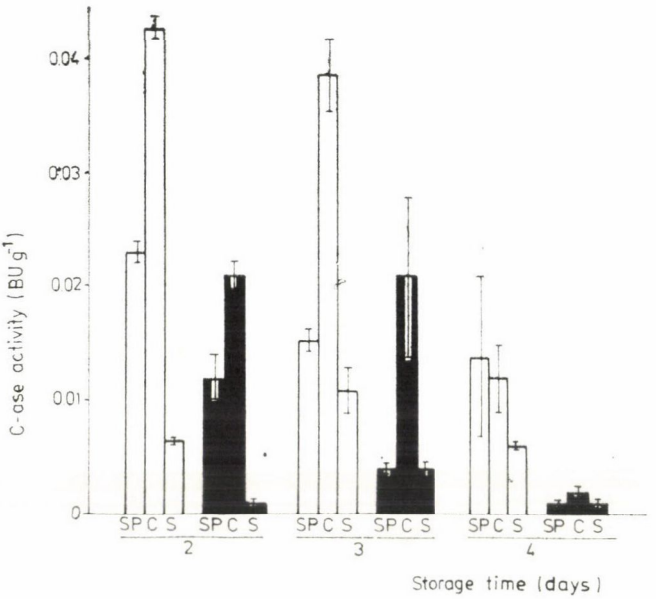


Fig. 14. C-ase activities of mushroom parts 2, 3 and 4 days after irradiation. For details see Fig. 13

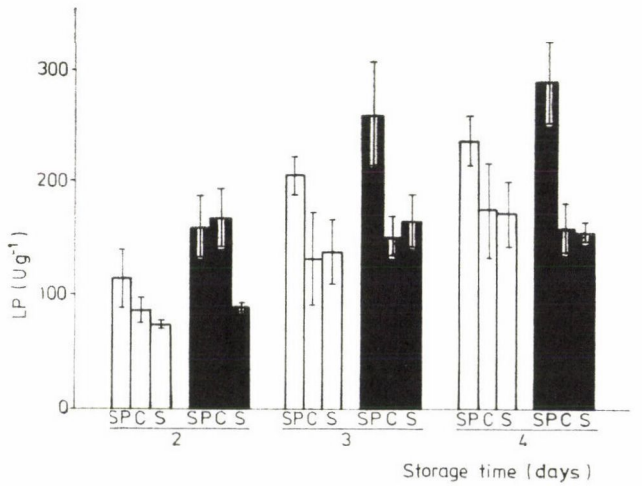


Fig. 15. LP contents in mushroom parts after irradiation and in control samples. For details see Fig. 13

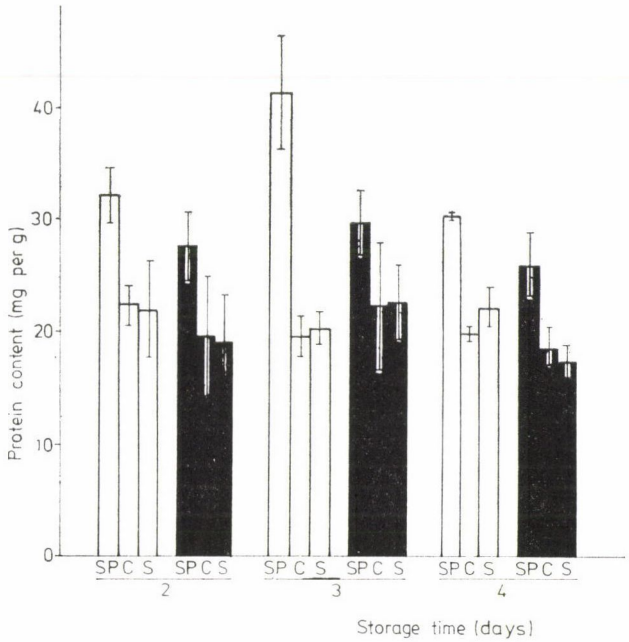


Fig. 16. Protein contents in mushroom parts after irradiation (■) and in control (□) samples. For details see Fig. 13

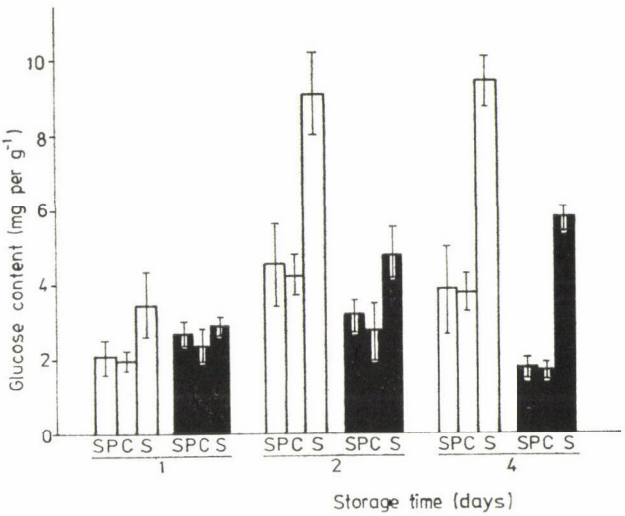


Fig. 17. Glucose contents in mushroom part homogenates of controls and in samples of 1, 2 and 4 days after irradiation

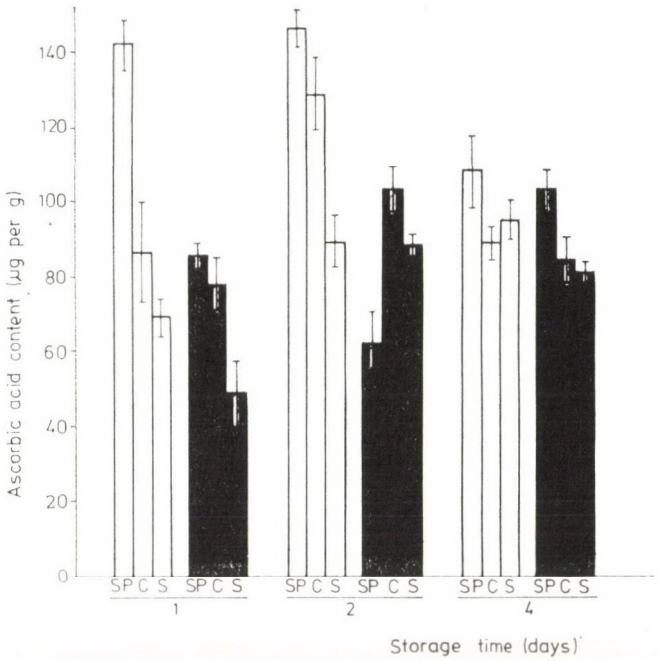


Fig. 18. Ascorbic acid contents of mushroom parts. For details see Fig. 17

lower in the irradiated sample than in the control on the 2nd day and in all parts of the mushroom on the 4th day. The ascorbic acid data in Fig. 18 reveal significantly lower values in the irradiated cap, mainly on the 1st and 2nd days.

## 3. Discussion

We have long been dealing with the antioxidant enzyme activities, lipid peroxidation, and glucose, ascorbic acid and pectin contents of different foodstuffs. We have now studied and compared these parameters in three basic materials of the food industry: onions, potatoes and mushrooms, under normal conditions and after irradiation. Our main aim was to compare the oxidative changes in different parts of these materials.

The metabolic consequences and chemical changes occurring in onions as a result of gamma-irradiation have been dealt with by CHACHIN and co-workers (1972) and MAHMOUD and co-workers (1978) and by the authors referred to in their publications. Convincing graphical representation of the effect of irradiation are also to be found in the IAEA reports and the discussions in the supplements (ANON., 1981; DE ZEEUW, 1976; ELIAS, 1976; SUNDARAM, 1976 and VAS, 1976a, b).

Since potatoes fulfil a much more general role as a fundamental foodstuff, there is a much larger literature on the radiation-treatment of potatoes. Here, too, we shall highlight only a few articles and the references to be found in them. As an example, MACDONALD (1967) studied the role of oxygen tension in the respiration of potato slices, and observed that this depends on the age of the slices and on the temperature, i. e. overall on the diffusion of air. The metabolic consequences of irradiation in potatoes were investigated by OGAWA and co-workers (1968, 1969) and KODENCHERRY and NAIR (1972). KESAVAN (1978) made a much more general study of the question of radiation treatment, and concluded that irradiated potatoes do not suffer a deterioration in taste and display no mutagenic properties.

The most wide-ranging work on the oxidative changes is probably that of KÖTELES and KUBASOVA (1982). Information on a number of problems is provided by a survey of their publication, which deals with the oxidative changes induced in the cell membranes by ionizing radiation. The membranes are the primary targets of radiation, which is the reason why the publication is stressed.

Mention may also be made of the work of SZÓRÁD and SZABÓ (1981) on the physiological effects of ionizing radiation. They survey the investigations carried out in Hungary, and outline the future perspectives. The interdisciplinary principle they emphasize is well worthy of consideration in relation to the ionizing irradiation of foodstuffs, and the relatively cheap enzymatic methods could replace many expensive measurements with large instrumentation.

Our own investigations on quantitative comparisons of the antioxidant enzyme activities (SOD, P-ase and C-ase), LP and glucose, ascorbic acid and pectin contents in irradiated and control onions, potatoes and mushrooms resulted in the demonstration of a characteristic oxidative dynamism which had previously not been studied. We do not know how the oxidative enzyme and LP changes we have described will later influence the taste of these foodstuffs, their external appearance and their other properties, but we have established that these changes persist for a long time. It also appears that new physiological and metabolic pathways appear in the irradiated plants, differing from those in the untreated (control) foodstuffs.

The objective evaluation of such findings necessitates further complex investigations.

*

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# VIABILITY OF FOOD POISONING MICROORGANISMS IN EGYPTIAN SALADS

ZAKIA A. HELMY, AFAF ABD-EL-BAKEY and Z. Y. DAW

Microbiology Department, Faculty of Agriculture, Cairo University, Cairo–Giza. Egypt

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Microorganisms of public health significance, i. e. faecal coliform, *Str. faecalis*, *Staph. aureus*, *S. typhimurium* were inoculated into three different types of laboratory-made salads (mayonnaise, tehina and green vegetable) of different pH and salt content and their growth trends were followed either at 3 °C or 30 °C.

The faecal coliform, being inoculated into each of the laboratory made salads, could not initiate growth in any of the salads even under the more suitable conditions, however, in the tehina salad of pH 5.0 late slight growth was observed at room temperature. Being in association with *Str. faecalis* organism, the latter was able to dominate the population at 30 °C in both the tehina and the green salads.

In the mayonnaise of pH 5.0 and at room temperature *Str. faecalis* showed a slight growth during the 24 h of incubation, but not thereafter. The initial condition of the salads tested permitted the growth of *Str. faecalis* and not that of faecal coliforms.

At neither condition could *Staph. aureus* grow in the tehina or in the mayonnaise salads, however, it could keep alive for longer time in the refrigerator and in less acidic salads.

Similar results were obtained for salmonellae growth.

**Keywords:** public health, coliforms, green salads

In a survey study including the determination of the microbial content of the Egyptian market salads, i. e. tehina (ground sesamy), mayonnaise and green vegetable salads (HELMY et al., 1984), it was found that the initial level of contamination ranged from $5.3 \times 10^5\ g^{-1}$ in tehina salad, to $8.5 \times 10^7\ g^{-1}$ in mayonnaise salad. The groups of organisms which are encountered in high counts in these salad samples were of hygienic significance, i. e., coliforms including the faecal types and, to a lesser extent, faecal enterococci as well as salmonellae.

Coliforms, although regarded as part of the normal flora of the human intestinal tract and that of many animals, yet enteropathogenic serotypes have been reported to be implicated in human diarrheal diseases and food poisoning outbreaks (SOJKA, 1973; SACK, 1975 and KLIPSTEIN et al., 1977).

Since salads are generally consumed raw, they can act as vehicle for transmitting pathogenic enteric diseases. Also, food poisoning and infection due to contamination of salad have been reported by MEYER and OXHOJ (1964).

An outbreak of salmonellosis occurred when a non-acid mayonnaise was the vector (ANON, 1976; FOSTER, 1976). FOWLER and FOSTER (1976) found that

26% of the mixed green salads, 28% of the green salads contained faecal coliform while *Staph. aureus* was found in 8% and 14% of the different samples, respectively.

So, the aim of the present work is to throw some light on the behaviour of certain organisms of food poisoning and pathogenic concern, when being inoculated in the different salads of different pH levels, NaCl concentrations and stored at different temperatures.

## 1. Materials and methods

Three experiments were carried out to determine the viability of pathogenic indicator organisms, e. g. faecal coliform and *Str. faecalis* as well as pathogenic organisms, e. g. *Staph. aureus* and *S. typhimurium* in salads.

Representative strains from each of faecal coliform, *Str. faecalis*, *Staph. aureus* and salmonellae were previously isolated from salad samples (HELMY et al., 1984) and inoculated in laboratory made mayonnaise, tehina and green vegetable salad samples.

### *1.1. Effect of pH and storage temperature*

Batches of mayonnaise and tehina salad (1% salt) were adjusted either to pH 4.5 or/and 5.0, and seeded with a 24 h broth culture from each of the previously mentioned strains to give an initial concentration of approximately $10^6$ cells per $cm^3$. Each batch was divided into two flasks and incubated at 3 °C and 30 °C, respectively.

### *1.2. Effect of salt concentration*

Tehina salad of pH values of 4.5 and 5.0 were supplemented with salt in concentrations of 1, 2, 3, 4 and 5% and inoculated with faecal coliform ($10^6-10^7$ cells per g) and stored at room temperature.

### *1.3. Behaviour of faecal coliform in association with* Str. faecalis

Both organisms were inoculated at a level of $10^3$ cells per g in tehina salad (pH 5.2 and 1% NaCl) or at a level of $10^6$ cells per g in green vegetable salad (pH 4.5 and 1% NaCl) and stored either at 3 °C or/and at 30 °C.

Samples were taken at different intervals according to the salad examined. Counts of faecal coliform and *Str. faecalis* were determined according to the specifications of COLIFORM SUB-COMMITTEE (1949) and HAJNA and PERRY (1943), respectively, while *S. typhimurium* were counted on Brilliant Green

Agar (Bacto B. 285) and *Staph. aureus* were estimated on *Staphylococcus* medium No. 110 (CHAPMAN, 1945).

Samples were also chemically analysed for total chlorides according to the method applied by JOFFEE (1942) and pH was measured using a pH meter.

## 2. Results

### *2.1. Viability of pathogenic indicator organisms in salads*

*2.1.1. Faecal coliforms.* a) Mayonnaise salad: The growth curves of faecal coliforms in mayonnaise samples of pH 4.5 and 5.0 stored at 30 °C or 3 °C are illustrated in Fig. 1. Growth of the organism was not observed even at the most suitable temperature. However, the organism was viable for a greater length of time in the less acidic salad, as well as in the mayonnaise kept in the refrigerator (the survival time was 120 h), while, when the mayonnaise was stored at 30 °C, no viable cells remained after 48 h and 120 h in the samples of pH 4.5 and 5.0, respectively.

b) Tehina salad: The growth trend of faecal coliform in tehina samples of pH 4.5 and 5.0, supplemented with different concentrations of salt, at room temperature, is shown in Figs. 2 and 3. The lower pH value, either in the unsalted control or with any of the salted samples, greatly antagonized the growth of the organisms, however the organism could resist, surviving for more than 72 h

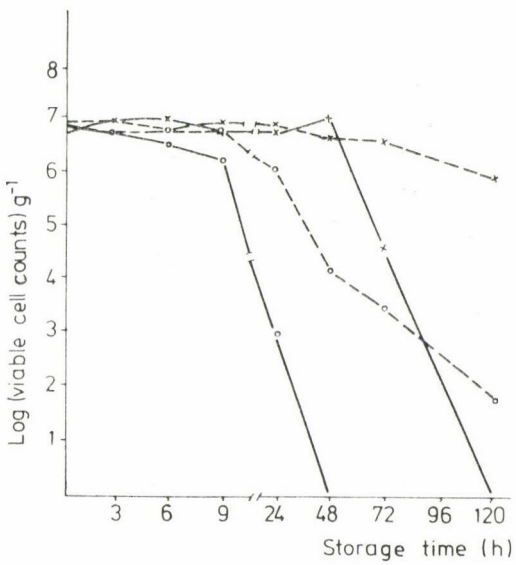


Fig. 1. Growth of faecal coliforms in mayonnaise samples of different pH values at 30 °C and 3 °C. o——o = pH 4.5, 30 °C; o·····o = pH 4.5, 3 °C; ×——× = pH 5, 30 °C; ×·····× = pH 5, 3 °C

in all of the cases. On the other hand, with pH 5.0 and in the samples of low salt concentration (0, 1 and 2% salt), slight growth was observed after a long lag period extending for 24 h.

c) Green vegetable salad: Growth trend of faecal coliform in the green vegetable salad stored at 30 °C and 3 °C is shown in Fig. 4. It was obvious that no growth was obtained in salad samples either at 30 °C or at 3 °C. Beside being

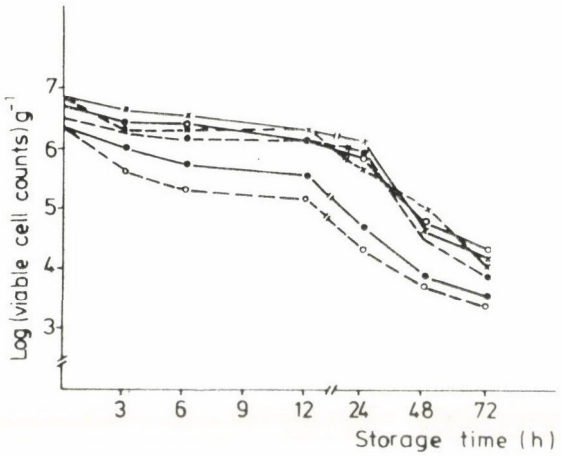


Fig. 2. Growth trend of faecal coliforms in tehina samples of pH 4.5 at different concentrations of salt (○——○ = 0%, ×——× = 1%, ×·····× = 2%; ●·····● = 3%; ●——● = 4%; ○·····○ = 5%) at room temperature

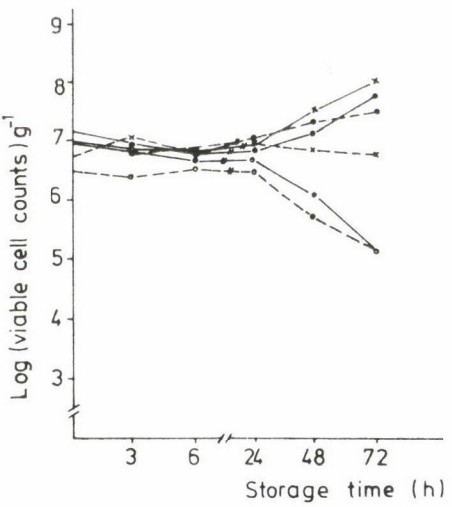


Fig. 3. Growth trend of faecal coliforms in tehina samples of pH 5 at different concentrations of salt (●——● = 0%; ×——× = 1%; ●·····● = 2%; ×·····× = 3%; ○——○ = 4%; ○·····○ = 5%) at room temperature

a matter of acidity, it was found that apart from lowering the pH, acetic acid has an inhibitory or even lethal effect on some coliforms (BROWNLIE & GRAU, 1967; GOEPFERT et al., 1968; GOEPFERT & HICKS, 1969).

*2.1.2. S. faecalis.* The growth curves of *S. faecalis* in the mayonnaise samples adjusted to pH 4.5 and 5.0 and stored at 30 °C and 3 °C, respectively, are illustrated in Fig. 5.

The organism could grow significantly and reached high numbers ($2.5 \times 10^8$) after 24 h of storage at 30 °C in the sample of pH 5.0, then a slow

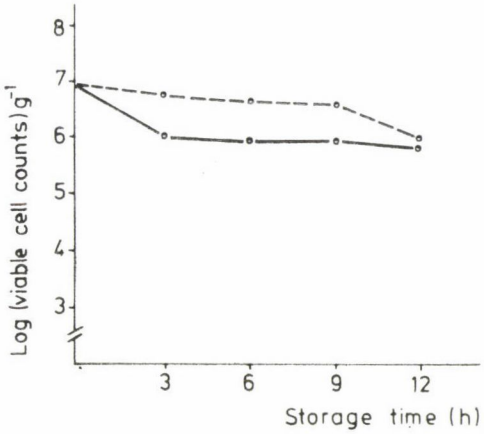


Fig. 4. Growth of faecal coliforms in green vegetable salad stored at 30 °C (○·····○) and 3 °C (○——○)

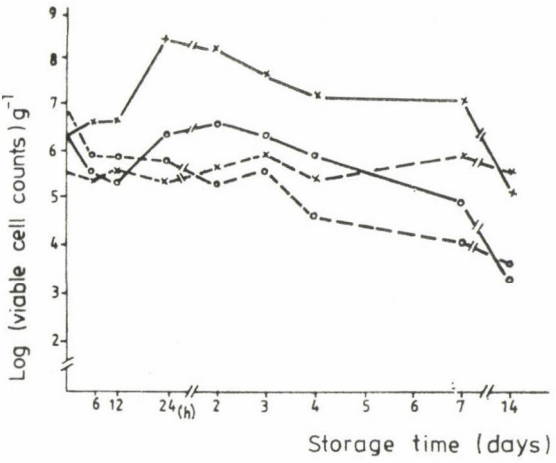


Fig. 5. Growth of *Str. faecalis* in mayonnaise samples of different pH values at 30 °C and 3 °C. ×·····× = pH 5, 3 °C; ×——× = pH 5, 30 °C; ○·····○ = pH 4.5, 3 °C; ○——○ =pH 4.5, 30 °C

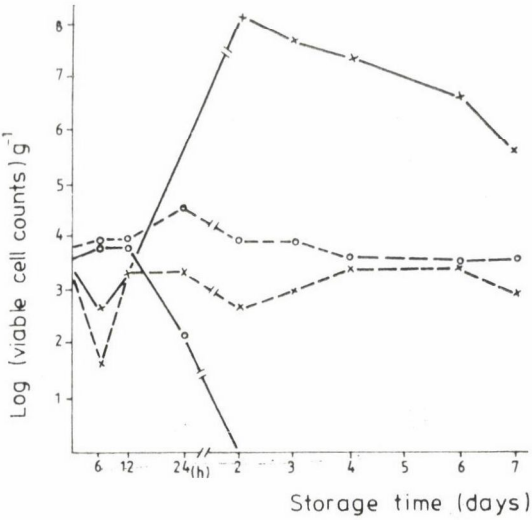


Fig. 6. Growth of faecal coliforms in association with *Str. faecalis* in tehina samples stored at 30 °C and 3 °C. ×——×= *Str. faecalis* 30 °C; ○·····○= faecal coliform 3 °C; ×·····×= *Str. faecalis* 3 °C; ○——○= faecal coliform 30 °C

death rate occurred, followed by a sharp decline. However, when the medium was more acidic, partial inhibition was observed during the first 12 h then a slight growth for 2 days was obtained, and thereafter similar death trend as that detected in the medium of pH 5.0 was observed.

At lower temperature, marked inhibition was obtained with both pH values, although the duration of the death phase was longer.

*2.1.3. Faecal coliform in association with* Str. faecalis. The growth of faecal coliform in association with *Str. faecalis* in the tehina and green vegetable salad samples stored either at 3 °C or 30 °C is shown in Figs. 6 and 7.

— Tehina samples:

When salad samples were stored at 30 °C, the *Str. faecalis* markedly grew after a lag period of 6 h. The maximal counts were obtained at the end of 2 days and were found to be $1.5 \times 10^8$ $g^{-1}$. However, faecal coliforms could not resist these conditions and were almost completely inhibited in 2 days.

On the other hand, *Str. faecalis* did not show growth at 3 °C, while the coliform type predominated through the first 24 h of incubation. So, at low incubation temperature, the two organisms could keep alive for more than 168 h and, thus, this salad can be considered as a food poisoning agent, providing that the contaminants were of toxical nature.

— Green vegetable salad:

Enterococci were capable of growth after a lag period of 12 hours. Thereafter, the organisms greatly increased in numbers and continued to grow

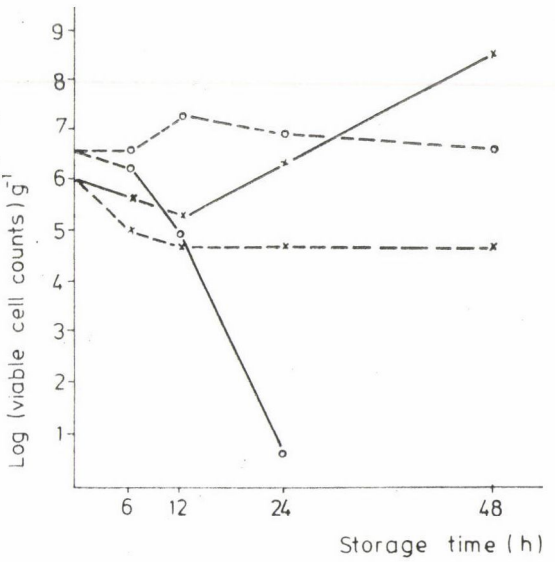


Fig. 7. Growth of faecal coliforms in association with *Str. faecalis* in the green vegetable salad samples stored at 30 °C and 3 °C. ×——×= *Str. faecalis* 30 °C; ○·····○= faecal coliform 3 °C; ×·····×= *Str. faecalis* 3 °C; ○——○= faecal coliform 30 °C

for 48 h. The faecal coliform, on the other hand, was mostly inhibited, thus no viable cells were recovered after 2 days. However, the rate of inhibition in associative growth was lower than that obtained with the organism alone under similar conditions, which may be a result of other antagonistic factors, particularly as the sharp declining phase was occurring mostly during the active growth period of *Str. faecalis*. At low temperature no growth was obtained for the enterococci while the faecal type of coliform showed a slight increase. This might be due to the psychrophilic nature of the organism which enables it to grow and dominate the population, in competition, through the first few hours.

### *2.2. Viability of pathogenic organisms in salads*

Batches of mayonnaise and tehina salad samples were adjusted to pH values of 4.5 and 5.0, inoculated with either *Staph. aureus* or *S. typhimurium* and stored at 30 °C or 3 °C. The growth curves are shown in Figs. 8, 9, 10 and 11.

*2.2.1. Staph. aureus.* The organisms failed to grow in the mayonnaise salad of pH 5.0 either when incubated at 30 °C or at 3 °C; however, their survival time lasted for more than 22 days. A late growth was observed in the sample of pH 5.0, stored at 30 °C, after 3 days, which probably may have been a sufficient lag to allow the selection of certain bacterial cells to grow under such conditions.

In the more acidic salad, *Staph. aureus* was completely inhibited after 24 hours in the samples stored at 30 °C and at 3 °C, respectively.

No visible growth was obtained in any of the tehina salad samples. However, the organisms stayed alive for a greater length of time at refrigerator temperature than at 30 °C. The rate of decline was also much slower in the

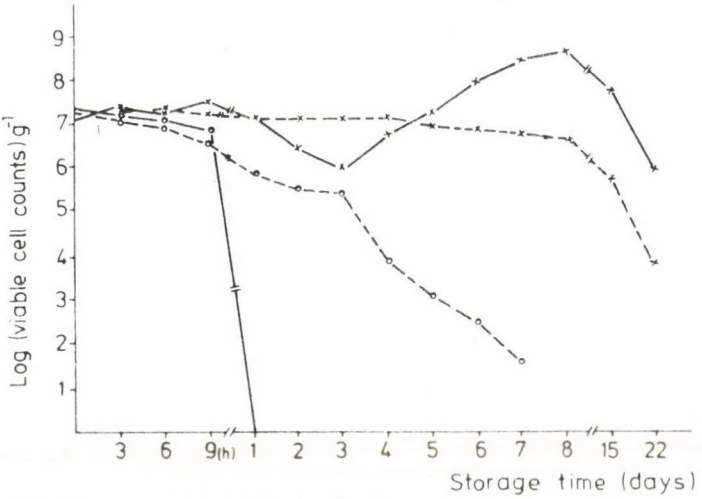


Fig. 8. Growth of *Staph. aureus* in mayonnaise samples of different pH values at 30 °C and 3 °C. ×——×= pH 5, 30 °C; ×·····× = pH 5, 3 °C; o·····o= pH 4.5, 3 °C; o——o = pH 4.5, 30 °C

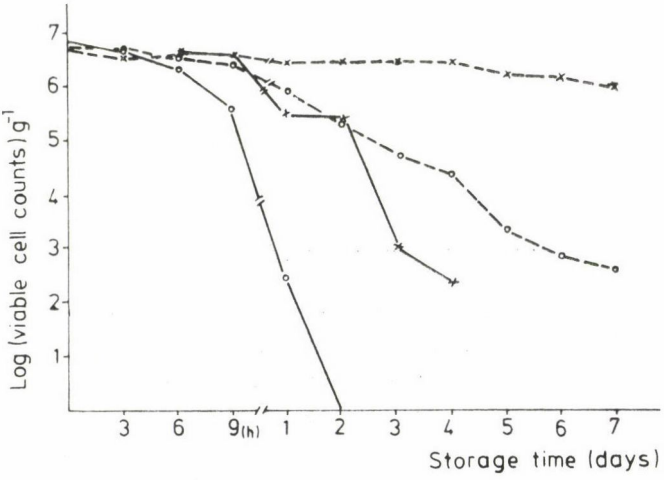


Fig. 9. Growth of *Staph. aureus* in tehina samples of different pH values at 30 °C and 3 °C. ×·····× = pH 5, 3 °C; o·····o= pH 4.5, 3 °C; 3 °C; ×——×= pH 5, 30 °C; o——o = pH 4.5, 30 °C

sample of higher pH values, and at 3 C than at 30 °C. Similar results were obtained by CHRISTIANSEN and KING (1971), when approximately $10^6 g^{-1}$ coagulase positive staphylococci were inoculated in each of chicken salad (pH 5.2) and ham salad (pH 4.8) and incubated at 4 °C and 37 °C. They stated that much slower decline was observed at 4 °C than at 37 °C.

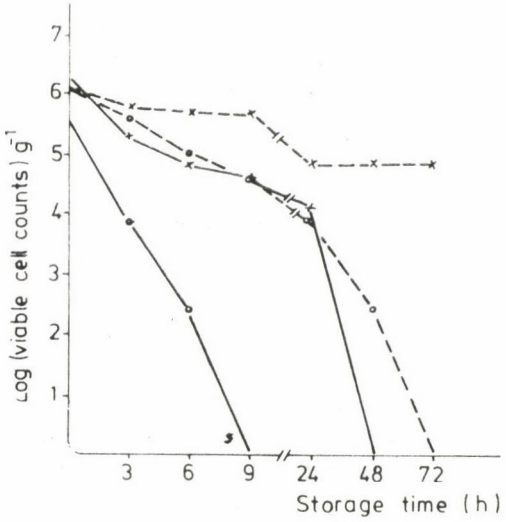


Fig. 10. Growth of *S. typhimurium* in mayonnaise samples of different pH values at 30 °C and 3 °C. ×------×= pH 5, 3 °C; ○------○ = pH 4.5, 3 °C; ×——×= pH 5, 30 °C; ○——○ = pH 4.5, 30 °C

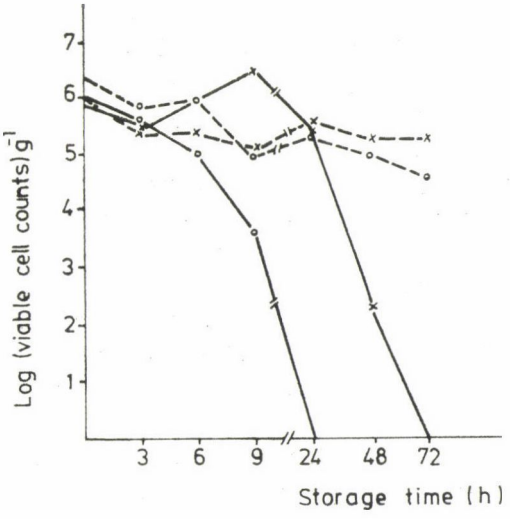


Fig. 11. Growth of *S. typhimurium* in tehina samples of different pH values at 30 °C and 3 °C ×------×= pH 5, 3 °C; ○------○ = pH 4.5, 3 °C; ×——×= pH 5, 30 °C; ○——○= pH 4.5, 30 °C

*2.2.2. Salmonella typhimurium.* Very effective inhibition was observed for the Salmonella growth in the different mayonnaise samples, indicating that the conditions were unsuitable for its propagation and viability. However, in the sample of pH 5.0, the organism could survive for more than 72 h at refrigerator temperature, a condition that commonly dominated during production and keeping of the commercial product.

In case of tehina salad, growth was not observed at either of the incubation temperatures, but the organism could survive for more than 72 h in great numbers at 3 °C. However, the organism declined much more rapidly at 30 °C.

## 3. Discussion

Survival or growth potential of the pathogenic organisms in the salads was of special concern. It is well known that mayonnaise is unfavourable for growth and survival of most bacteria, especially pathogens, primarily due to the low pH of the product (HENTGES, 1967; GOEPFERT & HICKS, 1969; CHUNG & GOEPFERT, 1970; KRAUSE, 1971; MATCHES & LISTOM, 1972). In the present study, the inoculated faecal coliform strain could not initiate any visible growth in either of the salads examined, if the acetic acid concentration was adequate. The bactericidal activity of acetic acid for typhoid and colon bacilli was reported by WINSLOW and LOCKRIDGE (1906), and for coliform by CHRISTIANSEN and KING (1971). The antimicrobial effect was found not to be solely due to pH but was probably a result of undissociated acetic acid molecules (LEVINE & FELLERS, 1940; HENTGES, 1967; MINOR & MARTH, 1972). In salads of lesser acidity such as tehina salad of pH 5.0 in the presence of low concentration of salt (1–2%), the organism grew significantly after a lag period of 24 h at room temperature. However, such growth was greatly inhibited when being in association with *Str. faecalis* in the tehina and green vegetable salads. *Str. faecalis*, on the other hand, actively grew and dominated the initial population. FOWLER and CLARK (1975) also found that enterococcus organisms were present in initial cell counts.

Apart from acidity, the other preservative system in mayonnaise depends on the dissolved solids that bind the available water (a. w.). So, the growth of *Str. faecalis* was not as vigorous in mayonnaise as in tehina or in green vegetable salads.

Although neither *Str. faecalis* nor the faecal coliforms started growth at refrigerator temperature, yet they survived much longer at 3 °C than at 30 °C. Similar observation was reported by HOLTZAPFFEL and MOSSEL (1968), who found that the death rate of Salmonella and *Staph. aureus* was faster in salads stored at 20 °C than at 9 °C.

*S. typhimurium* was found to be unable to grow in the mayonnaise samples. According to TROLLER (1973) since mayonnaise had a water activity of 0.93 (equivalent to 12% NaCl), salmonellae would not grow in it. On the other hand, the organism could slightly grow in the tehina salad of pH 5.0; this was probably a matter of water activity, as water generally was used to dilute such type of salad in preparation. Although *S. typhimurium* was rapidly killed in the salads stored at room temperature, it could survive much longer at refrigerator temperature. However, MATCHES and LISTON (1972) reported that cells of salmonellae stored at pH 4.0 died off rapidly at temperatures below 10 °C.

*Staph. aureus*, when being inoculated in tehina and mayonnaise salads of pH 4.5 and 5.0, was found not to be able to grow either at 30 °C or 3 °C, although the available water of mayonnaise would permit the organism's growth. But since pH and NaCl interact, mayonnaise was previously reported to be bactericidal to staphylococci (GRAM, 1957; BOVRE, 1958; SMITTLE, 1975). The inhibitory pH for *Staph. aureus* was found to be 5.0 and the lethal pH was 4.9 (LEVINE & FELLERS, 1940), a result which agreed with those obtained in both mayonnaise and tehina salads in the present work.

The production of enterotoxins depends on the growth of the organism. Since no *Staphylococcus* growth was observed in mayonnaise and tehina salads, therefore the probability of the presence of enterotoxins was nil. In addition, the conditions in each of the stored salads examined from the point of view of pH value and available water, seemed not to permit enterotoxin production as was found by HOJVAT and JACKSON (1969), MCLEAN and co-workers (1968); MARKUS and SILVERMAN (1970); TROLLER (1971).

Thus, it can be concluded that the salads tested are capable of supporting the growth of certain types under favourable conditions. But, in general, when pH values were low and salads were refrigerated, very little, if any, microbial growth occurred. Microorganisms of public health significance, on the other hand, are found to be a problem which could be avoided by following good sanitary practices during preparation and storage of the foods.

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# THE EFFECT OF ALUMINIUM CONTAMINATION ON THE SENSORY PROPERTIES OF LIQUID FOODS

M. Böröcz-Szabó

Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary

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During processing foodstuffs get in direct contact with construction materials and wrapping materials made of aluminium. Since it was established that aluminium is not detrimental to health little effort was made to detect its effect on foodstuffs. The existence of a concentration value or concentration range was assumed above which the presence of aluminium can be detected by sensory evaluation. An assay was made to find the threshold value of concentration of aluminium contamination in liquid foods above which the presence of aluminium can be detected by a sensory panel. The main subject of our investigations was the effect of the corrosion product of 99.5% foundry aluminium on the sensory properties of foods, but the experiments were carried out with 99.99% aluminium, too. It was studied also whether the effect of aluminium on the sensory quality of foods, caused to be present by corrosion, can be modelled by the application of aluminium salts.

Both 99.5% and 99.99% aluminium, present in the food by way of corrosion, was found to be sensorially detectable at a relatively low level, while aluminium salts added to foods were detected only at a substantially higher concentration level. Changes at a significant level in the sensory properties of the various kinds of liquid foodstuffs studied were brought about by different amounts of the aluminium contaminant. Foods of higher protein content possess a lower tolerance to aluminium contamination than foods of high acid content. Every kind of food investigated was most sensitive to aluminium contamination caused by corrosion and tolerated the presence of aluminium salts better. Thus it became evident that the effect of aluminium contamination by corrosion cannot be modelled by aluminium salts added.

Threshold values of sensory detection were established in freshly prepared foodstuffs. The effect of aluminium contaminants during storage remains to be the subject of a further study.

**Keywords:** contaminations, sensory effects, aluminium packaging of liquid foods

During processing, but even more during storage foods might get into direct contact with aluminium construction materials. Equipment and utensils used in food production are mostly manufactured from foundry aluminium of 99.5% purity, in certain cases from Al–Mg or Al–Mn alloy and in particularly indicated cases from aluminium of 99.99% purity.

Bácskay (1949) established that aluminium was not at all detrimental to human health, at the level of general daily consumption (Underwood, 1977), because it leaves the digestive canal without being absorbed (Kolb, 1975). Aluminium hydroxyde is used even as a gastric buffer (Almagel $Al(OH)_3$–$Mg(OH)_2$). Probably this is why so little research was done on the effect of aluminium on foods (Koch & Breker, 1958; Széchenyi, 1964). However,

the corrosive effect of foods on aluminium was the subject of more investigations (DOMONY & LICHTENBERGER, 1957; BÖRÖCZ–SZABÓ, 1977).

Since aluminium in itself is a rather corrosion-sensitive metal its resistance to corrosion is due to the oxide layer formed on its surface. According to KUNZE (1976) the corrosion resistance of aluminium wrapping materials is mainly dependent on the thickness, structure and composition of this oxide layer. The symptoms accompanying the corrosion of aluminium not given surface protection treatment differ from those observed with varnished aluminium, or given another surface treatment, attacked through pores or other breaks.

Studying aluminium corrosion in relation to food the most important factors are the moisture content, consistency, pH, salt content, the quality and quantity of seasonings, however, it is a proven fact that the same kind of fruit originating from different places of cultivation or of different year of growth may show very different aggressivity.

KUNZE (1976) leaning on data in the literature reviewed the effect of certain food groups on aluminium wrapping materials. He found that the authors describing actual cases of corrosion did not give the necessary details to be able to draw definite conclusions. That is one of the reasons why the corrosive effect of a certain food is often judged by different authors in a controversial way.

KUNZE (1976) found that varnishing only retards the corrosion of aluminium wrapping materials but does not completely inhibit it. The retarding effect is prolonged in the case of meat products, thus varnished aluminium is suitable for the packaging of meat products.

Certain natural food components behave as inhibitors and may reduce the corrosive effect of highly acidic foodstuffs. Thus, e. g. the aggressivity of acetic acid can be reduced by the addition of starch and some colloids show a similar effect.

In order to be able to gather information on the amount of aluminium dissolved by corrosion it is essential to have a reliable method for the determination with sufficient accuracy of the aluminium content of foods.

The HUNGARIAN STANDARD (1977) specifies for the determination of aluminium content a spectrophotometric method. After wet ashing the dissolved aluminium, reacted with 8 hydroxy-quinoline forms a yellow complex the colour intensity of which is directly proportional to the aluminium content. Measurement is carried out in chloroform solution.

The same procedure was followed by ESCHNAUER (1964) for the determination of aluminium in wine.

SPANYÁR and KEVEI (1962) suggest the use of aurin tricarbonic acid to form complex with the dissolved aluminium and measure the colour by spectrophotometry.

Chrome Azurol S is a reagent suitable for the determination of aluminium. SRIVASTAVA and co-workers (1963) made a thorough study of this reagent. PAKALNS (1965) developed the method based on the spectrophotometry of the complex formed by aluminium with Chrome Azurol S.

Analytical methods based on the spectrophotometry of the colour of the metal complex are of rather high time and labour requirement and the accuracy of results and reproducibility is not in proportion with the labour requirement.

Of the up-to-date instrumental methods atomic absorption spectrophotometry is the one most widely used. This method permits the direct determination of metals without wet ashing and enhancement in many liquid foods. LÁSZLÓ and co-workers (1978) applied this method to determine the aluminium content of wine and found it rapid and relatively accurate.

Our first aim was to find the threshold value of aluminium concentration, dissolved in liquid foodstuffs, above which the presence of aluminium can be established by sensory evaluation. Apart from the aluminium contamination by corrosion we added aluminium in the form of salts into the liquid food and studied the effect on the sensory properties.

We selected those aluminium salts the anions of which may occur in foods. By comparing the effect of aluminium salts to that of aluminium dissolved in consequence of corrosion, we tried to find out whether the effect of the latter can be modelled by the use of aluminium salts.

## 1. Materials and methods

### *1.1. Materials*

*1.1.1. Foods used in the experiments.* The following liquid foods (food components) were used:

- distilled water gained from a glass distilling apparatus;
- sour-cherry stock juice, product of the Canning Factory, Nagykőrös;
- apple-juice concentrate, product of the Canning Factory, Budapest;
- pasteurized milk, HUNGARIAN STANDARD (1973), from the retail market;
- beer (Kinizsi brand), HUNGARIAN STANDARD (1980), from the retail market;
- red wine (Extra rubin brand), product of the Budafok Winery;
- white wine (Nagykáta fehér) product of the Budafok Winery;
- commercial liqueur (White butter-pear liqueur), product of the Budapest liqueur factory, National Enterprise of the Alcohol Industry.

*1.1.2. Aluminium salts used in the experiments.* The following aluminium salts were used: $K\,Al(SO_4)_2 \cdot 12H_2O$, $Al_2(SO_4)_3 \cdot 18H_2O$, $AlCl_3$. The reagents were of analytical quality.

*1.1.3. Construction materials dissolved by corrosion in the food product.* Foundry aluminium of 99.5%, and 99.99% as used in the food industry, were applied.

*1.2. Methods*

*1.2.1. Preparation of the liquid foodstuffs contaminated by aluminium.* Sufficient aluminium salt was dissolved to obtain an aluminium concentration expressed by a round figure. Usually, in the first step the dissolved aluminium salt produced an aluminium concentration of 100 mg $kg^{-1}$ or 100 mg $dm^{-3}$. Immediately upon dissolution the product was exposed to sensory evaluation by triangular test. If the sensory test proved the sample to be significantly different from the aluminium-free sample, the test was repeated with a sample of aluminium content reduced to one half. Reduction of the aluminium content by half was continued till the difference became non-significant. In tables containing the results the two concentrations are given in one of which the aluminium contamination was recognized significantly and in the other just not recognized.

If the aluminium contamination of 100 mg $dm^{-3}$ concentration was not sufficient to be recognized the procedure described above was reversed in the direction of increasing the aluminium content.

Since sour-cherry stock juice and apple concentrate are not consumed as such alcohol-free beverages were prepared from them. The sour-cherry beverage contained 300 $cm^3$ stock solution, 102 g sugar, 2.2 g citric acid made up to 1000 $cm^3$ with tap-water. This beverage had a solids content of $R_s = 15\%$ and a pH = 3.2.

The apple concentrate was diluted with tap water to contain a solids content of $R_s = 10–12\%$ and 2 g $dm^{-3}$ citric acid. The pH was 3.4.

The amount of aluminium salt as calculated was dissolved in these beverages and the sensory tests were immediately carried out.

The bottled beer, stored at 283–285 K, was opened prior to testing. Since after dissolving the calculated amount of aluminium salt the major part of the carbon dioxide escaped, it was eliminated from the control sample, too.

In order to be able to test the effect of corroded aluminium test plates of $50 \times 100$ mm were prepared from 2 mm thick aluminium plates of 99.5% purity. Their surface was polished to the degree of metal purity and they were defatted and kept for a predetermined period in the liquid food, at room temperature. The test plates were placed in the liquid food, interspaced with little glass rods, to permit the aggressive liquid to freely circulate between the plates. After the test plates had been removed from the liquid food the aluminium content was determined. In order to establish whether the aluminium content was above the sensorially observable threshold value the liquid food was subjected to

triangular test. If the difference between the control samples and the sample containing aluminium was significant the latter was diluted with the control and tested afresh. If the aluminium present was below the sensory threshold more test plates were kept for a longer period in the liquid food till the concentration limit was reached above which the presence of aluminium could be significantly established.

In case of the sour-cherry stock juice, since the sample to be tested is prepared by dilution, the aluminium concentration in the stock juice was determined after removal of the test plates. This stock juice was diluted to contain the amount of aluminium required by the determination. The sour-cherry beverage was prepared by using the control stock juice.

The pasteurized milk with the test plates and the control samples were kept for 24 h at 273–275 K. After removing the test plates the milk was immediately subjected to triangular sensory test. Simultaneously the aluminium concentration in the milk was also determined.

The beer was tested similarly to the milk.

*1.2.2. Determination of the aluminium content.* The liquid food samples containing aluminium were first wet-ashed with nitric acid–sulfuric acid–perchloric acid according to SPANYÁR and KEVEI (1961). The aluminium content was determined by PAKALNS' method (1965). The komplex formed with Chrome Azurol S was subjected to spectrophotometry.

*1.2.3. Sensory test.* In accordance with the German Standard (DEUTSCHE INDUSTRIE-NORM, 1974) the sensory value was determined by triangular test. The panel consisted of 10 members. Panel members were given slightly sweetened biscuits and salted sticks between samples.

Results of the tests were evaluated according to the above standard using the tables in the appendix. Significance at the 95% probability level is marked by one asterisk, 99% by two asterisks, and 99.9% by three asterisks.

## 2. Results

### *2.1. Effect of the aluminium ions on the sensory properties of distilled water*

The sensory evaluation of distilled water appeared to be rather difficult. Distilled water has a disagreeable taste in itself. Aluminium salts make it unpleasant in another way and to differentiate between two unpleasant tastes is difficult.

The aluminium salts dissolved sufficiently well, however, above a certain concentration made the liquid opalescent or precipitated in flakes. This did not disturb the triangular test because panelists recognized the presence of aluminium at a substantially lower concentration than the one causing opalescence. Aluminium dissolved as a salt and not as the product of corrosion was

Table 1

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of distilled water as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 20 | 9 | *** | 9 | * |
| | 10 | 6 | — | 5 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 20 | 9 | *** | 9 | * |
| | 10 | 4 | — | 3 | — |
| $AlCl_3$ | 50 | 10 | *** | 10 | * |
| | 25 | 5 | — | 5 | — |
| Corrosion product of foundry aluminium | 0.1 | 8 | ** | 7 | — |
| | 0.05 | 2 | — | 1 | — |
| Corrosion product of 99.99% purity aluminium | 0.43 | 7 | * | 7 | * |
| | 0.21 | 5 | — | 2 | — |

Notes:
Correct answer to Question 1 was given by panel members detecting the two identical samples
Correct answer to Question 2 was given by panel members scoring the aluminium containing sample lower
* significant at $P \geq 95\%$ probability level
** highly significant at $P \leq 99\%$ probability level
*** very highly significant at $P \leq 99.9\%$ probability level

Table 2

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials, on the sensory properties of sour-cherry beverage as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 250 | 8 | ** | 4 | — |
| | 100 | 3 | — | 2 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 500 | 8 | ** | 7 | — |
| | 250 | 3 | — | 2 | — |
| $AlCl_3$ | 500 | 9 | *** | 9 | * |
| | 250 | 6 | — | 6 | * |
| Corrosion product of foundry aluminium | 6 | 7 | * | 7 | * |
| | 3 | 6 | — | 4 | — |
| Corrosion product of 99.99% purity aluminium | 7.8 | 7 | * | 4 | — |
| | 5.4 | 0 | — | 0 | — |

For explanations see Notes to Table 1

recognized at a higher concentration than the corrosion product. In distilled water the corrosion product was identifiable at a significantly lower concentration.

Results of the triangular test of aluminium contaminated distilled water are given in Table 1.

### *2.2. Effect of the aluminium ions on the sensory properties of fruit beverages*

The colour of the fruit beverages was not changed under the given conditions by the presence of aluminium ions. The change caused in taste appeared to be rather bitter than metallic. Here again the contamination produced by the dissolution of aluminium salts was recognized at a substantially higher level than that produced by corrosion.

The effect of aluminium contamination caused by corrosion could not be established in the apple beverage, because of the low aggressivity of the apple juice concentrate the aluminium concentration remained under the threshold level even after a longer storage period. After dilution to fruit drink concentration the aluminium content was not significant and caused no change in the sensory properties, whatsoever. These data are, therefore, not given in the tables.

The results of the sensory tests are summed up in Tables 2 and 3.

### *2.3. Effect of the aluminium ions on the sensory properties of wine*

Aluminium salts in wine were recognized at the concentration of 50 mg $dm^{-3}$. Although the taste was only slightly bitter this was sufficient for the panelists, considered average consumers, to establish the difference at a significant level.

Table 3

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of apple beverage as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| $K\,Al(SO_4)_2 \cdot 12\,H_2O$ | 500 | 10 | *** | 9 | * |
| | 250 | 6 | — | 6 | * |
| $Al_2(SO_4)_3 \cdot 18\,H_2O$ | 250 | 8 | ** | 8 | * |
| | 100 | 5 | — | 4 | — |
| $AlCl_3$ | 100 | 7 | — | 6 | — |
| | 50 | 2 | — | 2 | — |

For explanation see Notes to Table 1

Aluminium ions as corrosion products were recognized at a level of significance at a substantially lower concentration, although the taste was also slightly bitter.

Results of the sensory tests by the triangular method are summed up in Tables 4 and 5.

Table 4

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of white wine as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 50 | 7 | * | 7 | * |
| | 25 | 3 | — | 3 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 50 | 7 | * | 6 | — |
| | 25 | 2 | — | 2 | — |
| $AlCl_3$ | 50 | 8 | ** | 6 | — |
| | 25 | 5 | — | 4 | — |
| Corrosion product of foundry aluminium | 15.2 | 7 | * | 7 | * |
| | 7.6 | 2 | — | 1 | — |
| Corrosion product of 99.99% purity aluminium | 9.18 | 9 | ** | 7 | — |
| | 4.59 | 2 | — | 1 | — |

For explanation see Notes to Table 1

Table 5

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of red wine as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 50 | 10 | *** | 9 | * |
| | 25 | 5 | — | 5 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 25 | 8 | ** | 8 | * |
| | 20 | 4 | — | 4 | — |
| $AlCl_3$ | 25 | 9 | *** | 9 | * |
| | 20 | 3 | — | 2 | — |
| Corrosion product of foundry aluminium | 14.8 | 9 | *** | 8 | * |
| | 7.4 | 4 | — | 4 | — |
| Corrosion product of 99.99% purity aluminium | 11.42 | 8 | ** | 7 | — |
| | 5.71 | 5 | — | 5 | — |

For explanation see Notes to Table 1

*2.4. Effect of aluminium ions on the sensory properties of liqueur*

The effect of aluminium salts appeared in a minute change of colour. Panelists observed a bitter, throat scratching aftertaste, some of them found it sour. Aluminium contamination caused by corrosion was found unanimously to be bitter, tart.

Results obtained by triangular test are shown in Table 6.

*2.5. Effect of the aluminium ions on the sensory properties of beer*

Both the added aluminium salts and aluminium corroded in the liquid changed the original taste of beer to give a product of totally different taste. A bitter taste, differing from that originating from hop formed when the aluminium contamination was present in a significantly observable concentration.

Aluminium as a corrosion product caused the clear beer to become opalescent.

Results obtained by triangular sensory test are summarized in Table 7.

*2.6. Effect of aluminium ions on the sensory properties of milk*

In contrast to the other liquid foods investigated milk became sweet, sour or salty by the added aluminium salts.

Aluminium contamination as a product of corrosion caused a similar sensation but at a substantially lower concentration.

Table 6

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of commercial "Butter pear" liqueur as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| $K\,Al(SO_4)_2 \cdot 12\,H_2O$ | 100 | 8 | ** | 8 | * |
| | 50 | 5 | — | 5 | — |
| $Al_2(SO_4)_3 \cdot 18\,H_2O$ | 150 | 9 | *** | 9 | * |
| | 100 | 6 | — | 6 | * |
| $AlCl_3$ | 100 | 7 | * | 7 | * |
| | 50 | 4 | — | 4 | — |
| Corrosion product of foundry aluminium | 5.4 | 7 | * | 7 | * |
| | 2.7 | 5 | — | 3 | — |
| Corrosion product of 99.99% purity aluminium | 3.45 | 7 | * | 5 | — |
| | 1.73 | 4 | — | 4 | — |

For explanation see Notes to Table 1

Results obtained in various milk samples by triangular test are given in Table 8.

In Tables 1 to 8 the concentration values are given obtained by triangular test in the various liquid foods as an effect of added aluminium salts and aluminium as a corrosion product.

To have a better view over the subject in Fig. 1 the same data are graphically presented.

Table 7

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of commercial beer as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 100 | 7 | * | 4 | — |
| | 50 | 5 | — | 3 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 50 | 7 | — | 4 | — |
| | 25 | 4 | — | 2 | — |
| $AlCl_3$ | 25 | 9 | *** | 7 | — |
| | 12.5 | 2 | — | 2 | — |
| Corrosion product of foundry aluminium | 2.4 | 7 | * | 7 | * |
| | 1.2 | 5 | — | 5 | — |
| Corrosion product of 99.99% purity aluminium | <1 | 7 | * | 7 | * |
| | — | 4 | — | 2 | — |

For explanation see Notes to Table 1

Table 8

*The effect of aluminium contamination caused by added aluminium salts and the corrosion products of aluminium construction materials on the sensory properties of pasteurized milk as established by triangular test*

Number of panelists: 10

| Aluminium compounds | $Al^{3+}$ concentration (mg $dm^{-3}$) | Number of correct answers to Question 1 | Level of significance | Number of correct answers to Question 2 | Level of significance |
|---|---|---|---|---|---|
| K $Al(SO_4)_2 \cdot 12\ H_2O$ | 50 | 7 | * | 7 | * |
| | 25 | 4 | — | 3 | — |
| $Al_2(SO_4)_3 \cdot 18\ H_2O$ | 50 | 7 | * | 7 | * |
| | 25 | 3 | — | 3 | — |
| $AlCl_3$ | 50 | 7 | * | 7 | * |
| | 25 | 4 | — | 3 | — |
| Corrosion product of foundry aluminium | 1 | 7 | * | 7 | * |
| | — | 5 | — | 4 | — |
| Corrosion product of 99.99% purity aluminium | <1 | 9 | *** | 9 | * |
| | — | 3 | — | 2 | — |

For explanation see Notes to Table 1

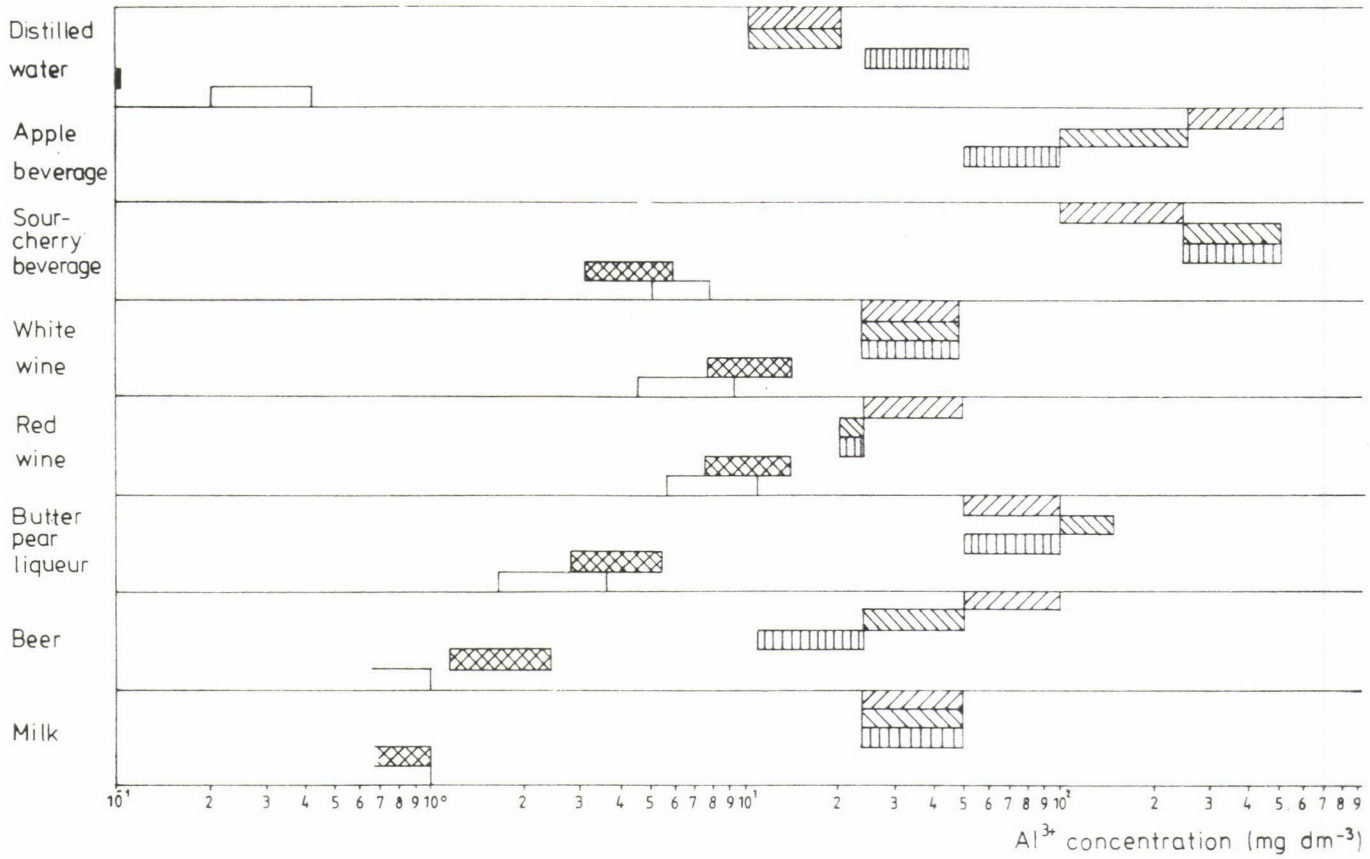


Fig. 1. Threshold values of detecting aluminium ion originated from added aluminium salts and the corrosion products of aluminium construction materials in liquid foods. ▨ : $K\,Al(SO_4)_2 \cdot 12\,H_2O$; ▨ : $Al_2(SO_4)_3 \cdot 18\,H_2O$; ▨ : $AlCl_3$; ▨ : foundry aluminium; ▭ : aluminium of 99.99% purity

## 3. Conclusions

*3.1. Changes in the sensory properties of distilled water*

The presence in distilled water of aluminium ions originating from aluminium salts can be detected at a significant level at about the same concentration as in wine, beer and milk. Of the liquid foods studied these are the ones which contain the highest percentage water, thus water may be considered their "solvent". The changes caused by aluminium ions and detected at a level of significance by triangular test were observed at about the same concentration in the "solvent" and in the "solutions".

Fruit drinks behaved differently, probably due to the presence of aroma substances and acids.

The presence of aluminium ions as a product of corrosion was detected in distilled water at the lowest concentration level of all the liquid foods studied.

In the course of this study the generally applied foundry aluminium of 99.5% purity was used at first. Since the presence of aluminium was detected at a substantially lower concentration than in the case of aluminium salts it seemed desirable to find out whether the changes in taste were caused really by the corrosion product. The changes in taste may have been caused by unidentified contaminants, present in foundry aluminium at a concentration of 0.5%, due to their more electronegative characteristic than that of the liquid food investigated.

Therefore it was decided to carry out the same experiments with aluminium of 99.99% purity, known as high purity aluminium. However, the presence of aluminium ions was recognized by the panel at the same or even at a lower concentration than in the case of 99.5% aluminium. Presumably the 0.5% contamination is not of metallic origin, thus, it does not dissolve in the food medium and therefore does not affect the sensory properties. But even if a part of this contamination were metal, this would be present in the form of minute inclusions of extremely small surface.

In the case of an anode of small surface (metallic contamination) and a catode of large surface (the rest of the test plate) a high current density forms at the anode, the contaminant dissolves and the process comes to an end. However, under the conditions of the experiments metal contaminants on or near the surface could only be dissolved, those deeper in the material could not.

*3.2. Changes in the sensory properties of fruit beverages*

It was found that changes in fruit beverages were not discernible by the naked eye. Aluminium as a corrosion product affects mostly the taste. This indicates the coating of the surface of all aluminium getting into contact with food materials (storage tanks, smaller vessels, packaging material) with durable,

pore-free plastic material, thereby saving the aluminium from corrosion and the foodstuff from contamination. From technological equipment provided with adequate coating even as low contamination as shown in the last four rows of Table 2 cannot get into the foodstuff.

It can be seen in the Table that the difference in the amount of aluminium dissolved in fruit juices from foundry aluminium and from high purity aluminium is insignificant.

*3.3. Changes in the sensory properties of wine*

The presence of aluminium ions in the wine may cause changes in its appearance, e. g. opalisation. However, other changes of sensory quality occur at lower aluminium concentrations, therefore the investigation of this phenomenon is of no interest.

Under the conditions applied colour changes did not become apparent. The presence of contamination was established on the basis of changes in the taste.

As regards the difference between the corrosion product of foundry aluminium and high purity aluminium the same applies to wine as to fruit juices.

*3.4. Changes in the sensory properties of liqueur*

The characteristic orange colour of the "White butter-pear liqueur" changed but slightly in the presence of aluminium, probably due to complex formation with the colouring substance. Certain effects on taste were masked by the high sugar content of the liqueur (40%). The fact that added $Al_2(SO_4)_3$ and $KAl(SO_4)_2$ were sensorially recognized at 150 mg $dm^{-3}$ and 100 mg $dm^{-3}$, respectively, is due to this. In contrast the throat scratching effect of aluminium as a corrosion product was observed at a much lower concentration. Just because liqueurs, due to their high sugar content are non too aggressive, rarely occurs that liqueurs are contaminated by aluminium. If, however, aluminium as corrosion product gets into the liqueur its effect upon the taste is recognizable at a relatively low concentration (5.4 mg $dm^{-3}$), therefore this has to be carefully avoided.

*3.5. Changes in the sensory properties of beer*

The threshold value of sensorially detectable aluminium contamination in beer originating from added aluminium salts is substantially higher than with $Fe^{2+}$ or $Fe^{3+}$ (BÖRÖCZ–SZABÓ, 1980). In contrast, aluminium contamination as a product of corrosion is, similarly to iron contamination, detectable at a much lower concentration. The presence of aluminium causes also opalescence,

cloudiness in beer, due probably to disadvantageous changes in the protein content.

Thus, it is evident that aluminium utensils in contact with beer have to be coated mainly to protect the quality of beer and only in the second order to protect the utensils from corrosion.

### *3.6. Changes in the sensory properties of milk*

The behaviour of milk was similar to the other foodstuffs studied. Added aluminium salts were sensorially detectable at much higher concentration levels than aluminium as a product of corrosion. The latter caused substantial change in the taste of milk at low aluminium concentration. While in other liquid foods the taste became bitter, in milk it became nauseatingly sweetish.

Aluminium as a corrosion product was detected at a significant level at as low concentrations as 1–2 mg $dm^{-3}$. This should be an incitement to give transport vessels and packaging materials an adequate coating.

Since, however, the shelf life is relatively short in the case of milk and thus the milk is in contact with aluminium during a limited period the danger of contamination is low.

Summing up the results it can be said that in the various liquid foods investigated aluminium contamination of different origin may be detected by triangular sensory test between different boundary values of concentration. Every liquid food studied was most sensitive to aluminium ions as the product of corrosion, independently from the aluminium content (99.5 or 99.99%) of the construction material used as test plate. Added aluminium salts were detected by the sensory panel at higher concentrations, therefore they are not suitable for modelling the effect of aluminium on the sensory properties of food.

To illuminate this phenomenon requires further investigation, however, it draws the attention to the need of providing proper coating in order to protect the foodstuffs against aluminium contamination and from deterioration of quality.

The sensory evaluations in the present study were carried out on freshly made samples. To find out the effect of aluminium ions during storage further experiments are intended.

*

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# SEPARATION OF, AND INVESTIGATION INTO THE PROPERTIES OF TRYPSIN AND CHYMOTRYPSIN FROM AN OVINE+CAPRINE PANCREATIC ENZYME PREPARATION

## I. SEPARATION OF THE ENZYMES BY AFFINITY CHROMATOGRAPHY

A. ZHIGZHIDDORZHIN[a,c], L. BOROSS[b] and L. VÁMOS-VIGYÁZÓ[a]

[a]Central Food Research Institute, H-1022 Budapest, Herman O. út 15. Hungary
[b]Department of Biochemistry, József Attila University of Szeged, H-6726 Szeged. Hungary

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The proteases trypsin and chymotrypsin of a commercial enzyme preparation, obtained by joint extraction and purification from sheep and goat pancreas, were separated by affinity chromatography on a CNBr-activated Sepharose 4B–soybean trypsin inhibitor column. Unbound protein (27–35% of total) was eluted with NaCl-containing phosphate buffer, pH 7.8. The same system, complemented with tryptamine, was used for the specific elution of chymotrypsin. Finally, trypsin was eluted by a salt-containing acetate buffer, pH 3.0. Tryptamine was removed from the pooled chymotrypsin fractions by gel filtration. The recovery of trypsin varied between 70 and 83% in the different runs. Trypsin fractions showed a slight chymotrypsin activity. Both these findings are in keeping with data of the literature. Chymotrypsin was obtained in a pure state, however, its recovery was low (up to 20%). Unbound proteins were recovered up to 88%. Autolysis as well as the proteolytic action of the enzymes might – at least partly – account for the losses of activity and protein content.

**Keywords:** ovine+caprine trypsin and chymotrypsin, affinity chromatography

Industrial enzymes are mainly produced by microorganisms. However, owing to its vast livestock (24 million domestic animals, made up to 70% by sheep and goat) Mongolia is able to cover its needs from the by-products of the meat industry. Up to 60 t of ovine and caprine pancreas are produced per annum in a sole vertical meet plant. This valuable source of various enzymes has been used since some years as raw material for the joint large scale production of an ovine + caprine trypsin–chymotrypsin preparation of pharmaceutical quality (ZHAMGANY & ZHIGZHIDDORZHIN, 1978; ZHAMGANY et al., 1979a, b). In order to extend the sphere of utilization of this preparation of the trade name of Pankipsin, the possibilities of its purification and separation into the two proteolytic components were investigated.

Out of the great number of communications available on pancreatic proteases relatively few (VITHAYATIL et al., 1961; BUCK et al., 1962; BRICTEUX-

[c]Present address: Research Centre of Enzymology and Microbiology, Meat Combinate Ulan-Bator. Mongolian People's Republic

GREGOIRE et al., 1966; TRAVIS, 1968; SCHYNS et al., 1969; PROSKURYAKOV, 1974) deal with those from sheep and none were found on those from goat or from a mixed preparation obtained from both sources. After having failed to separate trypsin from the chymotrypsin component of the given preparation by ion-exchange chromatography on CM-cellulose, a method successfully applied for separating bovine and porcine pancreatic proteases (KELLER et al., 1958; MAROUX et al., 1962; TRAVIS & LIENER, 1965; TRAVIS, 1967; LIENER, 1960) and recommended also for those of sheep (PROSKURYAKOV, 1974), the authors tried affinity chromatography (VESA, 1980). This biospecific method proved fit for the purpose. The present paper deals with the results of the preparative laboratory scale separation experiments, a second paper reports on some properties of the separated enzymes.

## 1. Materials and methods

### *1.1. The ovine+caprine trypsin–chymotrypsin preparation*

The preparation Pankipsin is produced at the Ulan-Bator Meat Combinate from sheep and goat pancreas by extraction, repeated precipitation with ammonium sulfate, subsequent desalting by dialysis and activation with calcium ions (NORTHROP et al., 1950). As attempts at crystallizing sheep pancreatic proteases have failed so far (BUCK et al., 1962), the preparation is marketed in a freeze-dried amorphous form as a mixture of trypsin and chymotrypsin.

### *1.2. Separation of the proteases by affinity chromatography*

Affinity chromatography was carried out at r. t., essentially according to the two-step procedure of the firm Pharmacia (ANON, 1974). Soybean trypsin inhibitor (STI) (Reanal, Budapest) was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala) (AXÉN et al., 1967) as follows: 25 g activated gel washed with 0.1 mol $dm^{-3}$ $NaHCO_3$ were passed through a Büchner funnel and stirred for 1 h with 15 $cm^3$ STI solution (20 mg STI dissolved in 1 $dm^3$ 0.1 mol $dm^{-3}$ $NaHCO_3$ buffer, pH 8.45, containing 0.5 mol $dm^{-3}$ NaCl). Subsequently, the unbound inhibitor was washed off with five times 30 $cm^3$ of the above buffer. The gel thus prepared contained about 1.5 g $dm^{-3}$ STI. This was then further washed protein-free ($A_{280} = 0$) with 100 $cm^3$ each of the coupling buffer and of 0.1 mol $dm^{-3}$ sodium acetate (pH 4.0) containing 0.5 mol $dm^{-3}$ NaCl and, thereafter, equilibrated with the buffer used for protein binding. Two buffer systems were tried for this purpose: *a)* 0.1 mol $dm^{-3}$ TRIS–HCl, pH 8.0, containing 0.2 mol $dm^{-3}$ KCl and 0.02 mol $dm^{-3}$ $CaCl_2$ and *b)* 0.1 mol $dm^{-3}$ phosphate buffer, pH 7.8, containing 0.5 mol $dm^{-3}$ NaCl.

An $1 \times 14$ cm glass column was filled with the gel, 10 mg ($A_{280}^{1\%} = 13.5$) Pankipsin solution were applied on top and elution of unbound proteins was started with one of the above buffers (*a* or *b*). After these had left the column ($A_{280} \sim 0$), specific elution of chymotrypsin was started with one of the eluting buffers complemented with 0.013 mol $dm^{-3}$ tryptamine hydrochloride. As this compound shows a strong absorbance at 280 nm, the elution of chymotrypsin was checked as follows: three to four fractions eluted from the Sepharose 4B–STI column were pooled and immediately applied on top of a column filled with Sephadex G 25 (Pharmacia, Uppsala) so as not to exceed 1/4 of the volume of the gel bed. Elution was performed at a rate of 0.5 $cm^3$ $min^{-1}$ with the buffer used to elute the inactive proteins from the Sepharose 4B–STI column.

After total elution of chymotrypsin from the Sepharose 4B–STI column, trypsin was eluted with 0.1 mol $dm^{-3}$ Na-acetate–acetic acid buffer, pH 3.0, containing 0.5 mol $dm^{-3}$ NaCl or 0.1 mol $dm^{-3}$ Na-acetate–HCl buffer, pH 3.0, containing 0.2 mol $dm^{-3}$ KCl and 0.02 mol $dm^{-3}$ $CaCl_2$. The elution rate was 0.25 $cm^3$ $min^{-1}$ and the fraction volume 3 $cm^3$.

Trypsin and chymotripsin activities as well as protein content were determined in the eluted fractions. These were then stored in frozen or freeze-dried state until further investigations.

### *1.3. Determination of enzyme activities and protein content*

Enzyme activities were assayed by specific methods. BAEE (N–α–benzoyl–L–arginine ethyl ester hydrochloride) was used as specific substrate for trypsin (SCHWERT & TAKENAKE, 1955) and BTEE (N–benzoyl–L–tyrosine ethyl ester) for chymotrypsin (HUMMEL, 1959). The assays were carried out exactly as described in the literature cited.

The change in absorbance ($A_{255}$) caused by 1 mg of enzyme protein in 1 min was adopted as activity unit.

The absorbance of the enzyme solutions at 280 nm was considered as protein content.

## 2. Results

Out of the two buffer systems used for elution the phosphate buffer system proved to be more advantageous with respect to both separation and yield of the separated enzymes. Figure 1 shows the elution pattern of the unbound proteins and trypsin.

The Figure is representative of another four elution patterns. The unbound proteins were eluted by the first 30 $cm^3$ of pH 7.8 buffer as a sharp protein peak. This contained, in the five runs performed, 27–35% of the total protein applied. The bulk of chymotrypsin was eluted with the next 95 $cm^3$ of pH 7.8

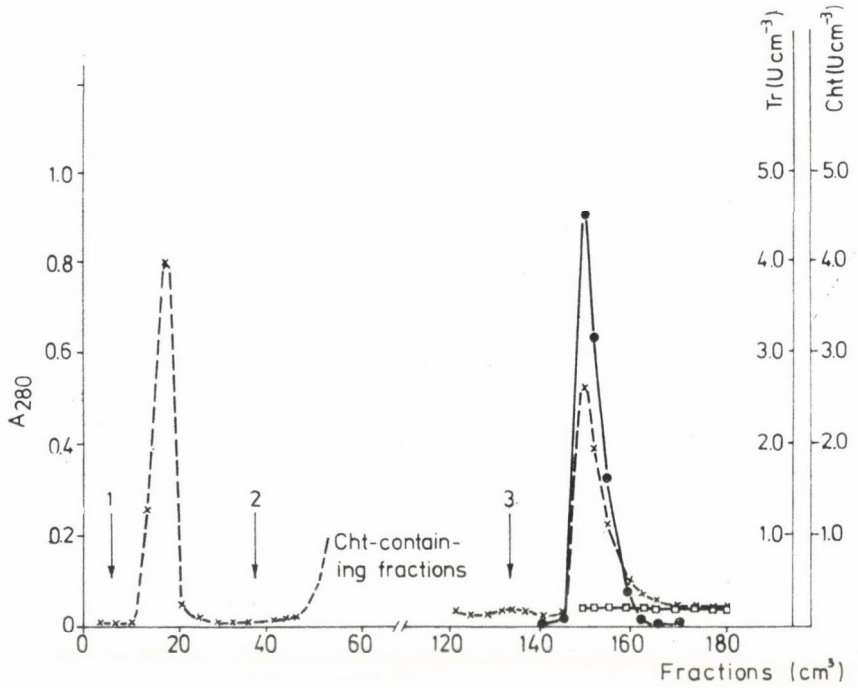


Fig. 1. Separation of ovine+caprine pancreatic proteases by affinity chromatography on a CNBr-activated Sepharose 4B–soybean trypsin inhibitor column. 1: start of elution with 0.1 mol $dm^{-3}$ phosphate buffer, pH 7.8, containing 0.5 mol $dm^{-3}$ NaCl; 2: start of elution with above buffer complemented with 0.013 mol $dm^{-3}$ tryptamine hydrochloride; 3: start of elution with 0.1 mol $dm^{-3}$ Na-acetate – acetic acid buffer, pH 3.0, containing 0.2 mol $dm^{-3}$ NaCl.
$A_{280}$ = absorbance at 280 nm; U = enzyme activity unit: change in absorbance at 255 nm as caused by 1 mg of enzyme protein in 1 min.
------: protein content; ———: enzyme activities; ●●●: trypsin; □□□: chymotrypsin

phosphate buffer, complemented with tryptamine. However, only the position of the chymotrypsin fraction could be marked in the elution diagram as tryptamine is a reversible inhibitor of the enzyme and thus the fractions had no measurable activity and the high own absorbance of tryptamine at 280 nm made protein assays of the enzyme fractions impossible. Elution with the tryptamine-containing buffer was carried on till practically no measurable activity and protein content were obtained in the pooled fractions passed through the Sephadex G 25 column (see Fig. 2). After elution of chymotrypsin had been finished, the trypsin content of the Pankipsin preparation bound to STI was eluted as a sharp peak with about 25 $cm^3$ of the pH 3.0 acetate buffer. The trypsin-containing fractions constituted, on the average, about 37% of the total protein and, in the five parallel runs, 70–83% of the total trypsin activity applied to the column. The elution pattern shows also that some of the chymotrypsin has been eluted together with the trypsin fractions.

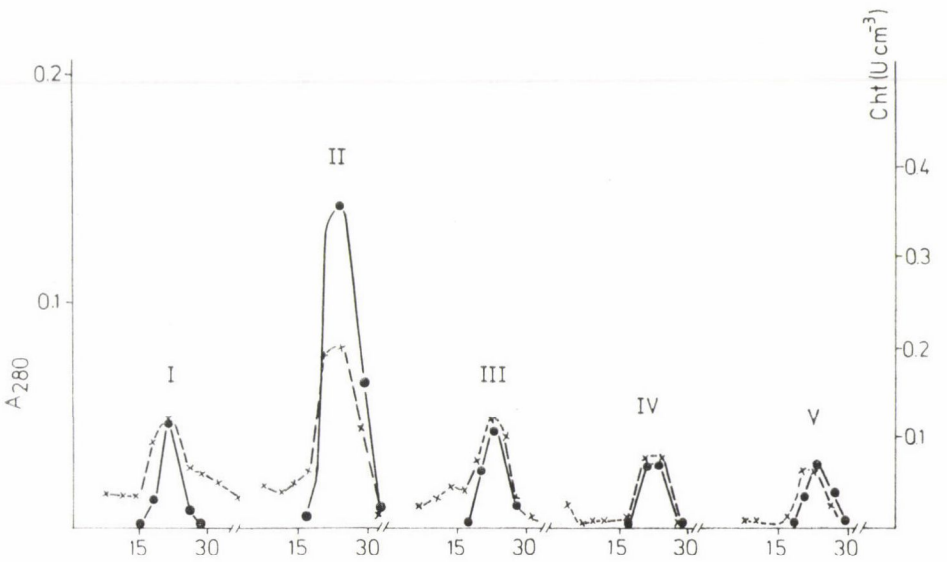


Fig. 2. Removal of tryptamine hydrochloride from chymotrypsin by gel filtration on Sephadex G 25. I–V: elution patterns of 3 pooled fractions each, eluted from the affinity column. For further signs and symbols see Fig. 1

Figure 2 shows the elution pattern of the pooled chymotrypsin fractions as obtained from the Sephadex G 25 column.

The Figure shows that the bulk of chymotrypsin activity liberated from tryptamine could be obtained in the first 60 cm$^3$ leaving the Sephadex G 25 column: 17–20% of the chymotrypsin present in the amount of Pankipsin applied to the affinity column could be recovered in the eluate of the Sephadex column, another 18% on the average, appeared as an impurity in the trypsin fractions.

Table 1

*Maximum recovery and increase in specific activity of ovine+caprine trypsin and chymotrypsin after separation by affinity chromatography*

| Recovery (%) | | | | | | Factor of increase in specific activity | |
|---|---|---|---|---|---|---|---|
| Protein ($A_{280}$) | | | | Enzyme activity | | | |
| Unbound | Tr | Cht | Total | Tr | Cht | Tr | Cht |
| 31 | 37 | 20 | 88 | 83 | 17 | 1.8<br>2.8[a] | —<br>2.0[a] |

Tr: trypsin
Cht :chymotrypsin
[a]: in the peak fraction
For details of separation see Figs. 1 and 2.

Protein recoveries in the inactive as well as proteolytic fractions, activity recoveries of trypsin and chymotrypsin as well as the increase in specific activities ($U/A_{280}$) in the peak fractions of the separation giving the highest yield of trypsin, are summarized in Table 1.

According to these data, the loss of total protein incurred during affinity chromatographic separation amounted to 12%, that of trypsin activity to 17%, while more than 80% of chymotrypsin activity were lost in the process. However, the figure given in the Table refers only to the chymotrypsin recovered as pure enzyme after elution from the Sephadex G 25 column. Nearly an equal amount of this enzyme was eluted together with the trypsin fractions.

## 3. Conclusions

The difference in the separation capacity of the two buffer systems applied might be due to the slight difference in pH, to the difference in composition and also to that in ionic strength (ANON, 1974). The effects of these factors are not yet clearly understood in affinity chromatography and make a certain amount of "trial and error" still indispensable in establishing the best separation scheme for a given mixture of enzymes. Changes in buffer composition, pH and ionic strength might be a further means to improve the separation and yield of the components of the mixed ovine+caprine trypsin–chymotrypsin enzyme preparation.

The losses of total and enzyme protein contents might be due to proteolysis during the separation. It is assumed that carrying out all the operations at temperatures around 0 °C would greatly reduce this type of losses. Practically no papers published so far on the separation and/or purification, by affinity chromatography, of trypsin and chymotrypsin make mention of yields or recoveries. In a recent paper dealing with the purification of trypsin, by affinity chromatography on a modified casein sorbent, (ŠAFAŘIK, 1983), recovery of trypsin activity was found to be about 75% and this figure was considered by the author as a satisfactory yield. In the present case trypsin recoveries of the same order or even somewhat higher were found.

Although the chymotrypsin content of trypsin could be reduced, by affinity chromatography, to about 1/10 of that found in the Pankipsin preparation, the trypsin preparation obtained in this way was not quite pure. A crystalline bovine trypsin preparation of Merck's (Darmstadt, GFR) analyzed in our laboratory, showed a slight activity on the BTEE substrate specific for chymotrypsin (ZHIGZHIDDORZHIN, 1983). Moreover, VITHAYATHIL and coworkers (1961) found in crystalline commercial bovine as well as amorphous ovine and porcine trypsin preparations treated in the same way, low values of chymotrypsin activity. The highest BTEE-splitting activity was found in the

ovine preparation, and this was similar to the value established by us. It seems, from the aforesaid, that complete liberation of trypsin from traces of chymotrypsin is not quite simple and requires probably several separation and purification steps of an efficiency similar to that of affinity chromatography.

Chymotrypsin could be obtained in a pure form, although at a very low recovery. As already mentioned, no data on yields in affinity chromatography were found in the literature for chymotrypsin. Thus it cannot be judged if the recovery achieved in the present study is acceptable. There may be several reasons explaining the low recovery: this enzyme is more susceptible to autolysis and/or proteolysis by trypsin, under the conditions of affinity chromatography, than trypsin; part of the enzyme is irreversibly inactivated by tryptamine. Any of these causes might be eliminated by appropriate alterations of the separation conditions (reducing temperature or selecting another affinity eluent instead of tryptamine). The reasons of why nearly half of the total chymotrypsin activity recovered was eluted together with trypsin, could be interpreted by assuming that affinity elution with the tryptamine-containing buffer had been discontinued too early. In view of the high dilution of the chymotrypsin fractions leaving the Sephadex column, a failure in detecting BTEE-splitting activities in pooled fractions following the first 180 $cm^3$ cannot be precluded. However, to be judged from elution patterns in Fig. 2, more complete elution would require extremely large volumes of eluent. From these results it would seem that chymotrypsin was bound very strongly to the STI. However, in a preliminary experiment using only the coupling buffer and the pH 3.0 buffer for elution from the affinity column, in the large peak containing both enzymes (separated but from the unbound proteins) the maximum of chymotrypsin activity somewhat preceded that of trypsin activity. This would indicate that trypsin was bound stronger to the STI.

It can be seen that there are several points to be clarified yet in order to improve the separation and purification of the two proteases from ovine + caprine pancreas. On the whole, it can be said, that this first attempt in the literature to separate and purify, by affinity chromatography, trypsin and chymotrypsin from the given source, resulted in a good recovery of the former enzyme with a degree of purity corresponding to that of purified commercial enzyme preparations and in obtaining – although with a lower yield – a pure chymotrypsin free of any proteolytic contaminations.

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# SEPARATION OF, AND INVESTIGATION INTO THE PROPERTIES OF TRYPSIN AND CHYMOTRYPSIN FROM AN OVINE+ +CAPRINE PANCREATIC ENZYME PREPARATION

## II. SOME CHARACTERISTICS OF THE SEPARATED PROTEASES

A. ZHIGZHIDDORZHIN[a], GY. HAJÓS and L. VÁMOS-VIGYÁZÓ

Central Food Research Institute, H-1022 Budapest, Herman O. út 15.
Hungary

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The isoelectric points (pI) and molecular masses ($M_r$) of trypsin and chymotrypsin, separated by affinity chromatography of a pharmaceutical pancreatic protease preparation from Mongolian sheep+goat, were estimated by high resolution thin-layer isoelectric focusing and thin-layer sodium dodecyl sulfate–polyacrylamide gel (SDS–PAG) electrophoresis with sensitive $AgNO_3$ protein staining. For trypsin the dependences of activity on pH and temperature, respectively, were established and heat stability of the enzyme was investigated, too. Results were compared to data obtained for the corresponding bovine (B) and porcine (P) enzymes and for commercial pancreatic proteases.

The pI-s of ovine+caprine (OC) trypsin and chymotrypsin were found to be 10.6 and 9.5, somewhat more alkaline and acidic, respectively, than the values found for B enzymes, and more alkaline than reported for ovine enzymes of other regions. Prolonged focusing revealed $\beta$- and $\alpha$-trypsin as separate protein bands with mobilities similar to those of the corresponding P, and different from those of B enzymes. This is contradictory to data from the literature. The electrophoretic pattern of the commercial OC protease showed three more bands of pI-s 6.2–7.2, 4.5–5.3 and 4.

A number of protein bands were obtained in SDS–PAG electrophoresis with all the enzyme preparations tested. The main fractions of trypsin of various origins had $M_r$ values 23 000–25 000; mercaptoethanol fragments of $\alpha$-trypsin of $M_r$ 13 000 and 10 000–11 000 as well as $\psi$-trypsin and autolysis products of $M_r$ $< 10\,000$ were also present. The OC chymotrypsin gave only one very distinct protein zone of $M_r$ 25 000 and a second faint band around $M_r$ 12 000, while B enzyme preparations contained additional bands in the range of $M_r$ 11 000–13 000. The comparison of IF and SDS–PAG electrophoresis results suggests the presence of $\alpha$-amylase (pI 5.3, $M_r$ 51 000–54 000) in the commercial OC protease.

In keeping with literature, no difference in pH optimum of activity was found between trypsins of various origin. The OC trypsin had a narrower temperature optimum and proved more heat stable than the commercial OC protease and B trypsin.

**Keywords:** ovine+caprine trypsin and chymotrypsin, isoelectric points, molecular masses, pH and temperature optima

In a previous paper (ZHIGZHIDDORZHIN et al., 1985) the results of the separation, by affinity chromatography, of trypsin and chymotrypsin of ovine + caprine origin were reported. The present paper deals with the estimation

[a] Present address: Research Centre of Enzymology and Microbiology, Meat Combinate, Ulan-Bator. Mongolian People's Republic

of the isoelectric points (pI) and molecular masses ($M_r$) of the two proteases as well as with the determination of the dependence of trypsin activity on pH and temperature, and of the changes in thermostability as caused by purification of this enzyme. Isoelectric focusing (IF) and electrophoretic techniques of very high resolution were applied for pI and $M_r$ estimation and all the results were compared to those obtained for the initial pancreatic enzyme preparation containing both proteases as well as for commercial bovine and porcine pancreatic proteases of various degrees of purity.

## 1. Materials and methods

### *1.1. Ovine+caprine trypsin and chymotrypsin*

The fractions of the separated and purified enzymes eluted in different runs from the affinity column (see Part I) were pooled and concentrated by freeze-drying. Appropriate dilutions were used for the analyses.

### *1.2. Commercial enzyme preparations used for comparison*

The following commercial enzyme preparations were used for comparison in the different analytical methods besides of Pankipsin, the source of the purified ovine + caprine enzymes (see Part I):

- Neopankreatin (Gedeon Richter, Budapest), a crude pancreatic enzyme complex of pharmaceutical quality;
- crystalline trypsin, bovine (Merck, Darmstadt, Art. 24 579);
- crystalline chymotrypsin, bovine (Reanal, Budapest, Art. 11 152);
- three-times crystallized α-chymotrypsin, bovine (Serva, Heidelberg, Art. 17 160);
- three times crystallized α-chymotrypsin, bovine (Sigma, Saint Louis, Art. C-4 129);
- crystalline α-chymotrypsin, bovine (Calbiochem, Lucerne, Art. 23 083);
- crystalline trypsin, porcine (Merck, Darmstadt, Art. 8 350).

### *1.3. Determination of enzyme activities*

The proteolytic activities of the preparations Pankipsin and Neopankreatin were determined by the Anson-method and expressed in hemoglobin units (Anon, 1966).

The specific ester substrates used for the determination of trypsin and chymotrypsin activities in the purified ovine+caprine as well as in the

crystalline commercial preparations, were BAEE and BTEE, respectively (SCHWERT & TAKENAKE, 1955; HUMMEL, 1959) (see Part I). In establishing the activity—pH and activity—temperature relationships as well as the heat resistance of trypsin, another specific substrate, DL-BAPA (N-α-benzoyl-DL-arginine-4-nitroanilide hydrochloride), was used (ERLANGER et al., 1961). With this latter substrate the change in absorbance at 410 nm ($A_{410}$) brought about by 1 mg of enzyme protein ($A_{280}$) was adopted as unit enzyme activity.

*1.4. Estimation of the isoelectric points*

The isoelectric points of the enzymes were estimated by isoelectric focusing (IF) in a pH-gradient 4–11 established by the ampholytes Servalyt T4–9 and Servalyt T9–11 (Serva, Heidelberg) on an agarose-sorbit thin-layer (HAJÓS & DELINCÉE, 1983). The procedure was as follows.

*1.4.1. Preparation of the supporting medium.* The 0.2 g Agarose IEF and Agarose C, respectively, and 2.5 g sorbit were made up with water to 25 $cm^3$, then boiled for 30 min in a water-bath till the solution was clear and free of bubbles. Subsequently, 0.84 $cm^3$ Servalyt T4–9 and 0.42 $cm^3$ Servalyt 9–11 were added, the mixture was poured on a 10×8 cm or 10×12 cm Gel Bond plastic plate (Marine Colloids Division, FMC Corporation, Bio Products, Rockland, Maine) straightened out previously and was kept overnight in the refrigerator.

*1.4.2. Focusing.* The layer thus prepared was placed into the horizontal flat-bed apparatus FBE 3 000 of Pharmacia (Uppsala); 3–15 $mm^3$ of the 10–25 g $dm^{-3}$ enzymes to be tested and standards, both dissolved in twice distilled water, were applied to the layer using a plastic pattern. The 0.5 cm broad strips of Whatman No. 17 filter paper dipped into 10 g $dm^{-3}$ $H_2SO_4$ and 20 g $dm^{-3}$ ethylene diamine, respectively, were placed on the anode and cathode ends of the gel plate. Focusing was performed under permanent cooling for 120 and 165 min, respectively, at 50–1000 V.

*1.4.3. Treatment of the plate after focusing.* The proteins were denatured (fixed) for 5–10 min in a solution of 17.3 g sulfosalicylic acid and 57.5 g trichloroacetic acid (TCA) in 500 $cm^3$. Thereafter the plate was washed twice in methanol for 10 min each, then pressed and dried in a hot-air stream. Staining of the proteins was carried out by shaking the plate for 10–20 min in a 1 g $dm^{-3}$ solution of Serva Blau G (Serva, Heidelberg). Subsequently, the plate was washed with a solution containing 700 $cm^3$ methanol and 200 $cm^3$ glacial acetic acid in 2 $dm^3$, until the background was clear, and then was dried.

The standard solution was composed of ferritin (Serva, Heidelberg, No. 21 318, 10 g $dm^{-3}$, pI 4.4), horse-myoglobin (Calbiochem, Lucerne, No. 475 922, 20 g $dm^{-3}$, pI 7.3), ribonuclease from bovine pancreas (Serva, Heidelberg, No. 34 390, 5 g $dm^{-3}$, pI 9.45) and cytochrome *c* (Reanal, Budapest, No. 0323,

20 g $dm^{-3}$, pI 10.65). The pI-s of the proteases were estimated by comparison of their locations on the electrophoretogram with those of the standards run on the same plate.

*1.5. Estimation of the molecular masses*

The molecular masses of the proteases were estimated by SDS–PAG electrophoresis according to WEBER and OSBORN (1969) in a flat-bed system using a 10% (m/v) gel containing urea. The proteins were dissolved in a 0.01 mol $dm^{-3}$, pH 7.2 phosphate buffer containing SDS and mercapto ethanol. Electrophoresis was carried on for 1 h at 50 V and r. t. and, subsequently, for 225 min at 100 V and 5 °C. The separated proteins were denatured with 200 g $dm^{-3}$ TCA and stained with $AgNO_3$ (MERRILL et al., 1982).

Proteases and standard proteins were diluted to 0.5 g $dm^{-3}$ and applied in amounts of 10–15 $mm^3$. The components of the standard solution (with $M_r$ values in brackets) were cytochrome *c* (Reanal, Budapest, No. 0323) (11 700), ribonuclease (Serva, Heidelberg, No. 34 390) (13 600), alcohol dehydrogenase (Serva, Heidelberg, No. 12 081) (41 000) and bovine serum albumin (Phylaxia, Budapest, No. 771 07) (68 000). Molecular masses were estimated by comparing the locations, in the electrophoretogram, of the proteases with those of the standard proteins.

*1.6. Determination of the temperature optimum of activity of the ovine+caprine trypsin*

Enzyme activities were assayed in the temperature range of 30–70 °C. The solutions of enzyme and DL-BAPA substrate (the latter prepared according to ERLANGER et al., 1961) were pre-heated to the temperature desired. Reaction mixtures containing 2 $cm^3$ substrate and 1 $cm^3$ enzyme solution were incubated up to 5 min at the temperatures selected. After the reaction periods desired three reaction mixtures each were removed from the water bath, (at 0.5- or 1-min intervals), 1 $cm^3$ each of 30% (v/v) acetic acid was added to stop the reaction and absorbance of the solutions was read at 410 nm in a spectrophotometer (type Spektromom 195, Hungarian Optical Works MOM, Budapest) against a blank treated in the same way. The composition of the latter was identical with that of the reaction mixture except that it contained enzyme previously inactivated with 30% (v/v) acetic acid. Enzyme activity was calculated by linear regression analysis from the three parallel measurements performed after each reaction period.

*1.7. Determination of the pH-optimum of activity of the ovine+caprine trypsin*

Measurements to determine the pH-optimum of trypsin activity were carried out in the pH-range of 7–9 on DL-BAPA substrate. This was prepared in 0.05 mol $dm^{-3}$ TRIS-buffers containing 0.02 mol $dm^{-3}$ $CaCl_2$. The pH values of the buffers were 7.0; 7.8; 8.13; 8.66 and 9.01. Measurements were carried out at r. t. The absorbances of the reaction mixtures containing 2 $cm^3$ substrate and 1 $cm^3$ enzyme solution were read at intervals up to 5 min against a blank containing the corresponding buffer instead of the enzyme solution. Measurements were performed in triplicate and activities calculated as above.

*1.8. Heat inactivation of trypsin*

The enzyme solution (pH 8.0) was held for 10 min at 60, 65 and 70 °C, respectively. After quick cooling, residual activities were assayed on DL-BAPA substrate at r. t.

Heat treatment was carried out as follows: 1.8 $cm^3$ pH 8.0 TRIS-buffer were preheated in test tubes for 4 min to the temperature desired, 0.2 $cm^3$ enzyme solution were added and the mixture was kept in the water bath for 10 min. At the end of this period the test tubes were cooled in ice-water to 25 °C. Heat treatments were performed in triplicate, residual activities were measured at r. t. as described in para. 1.7 and expressed as % of the activity of the untreated sample.

## 2. Results

*2.1. Trypsin and chymotrypsin activities of some pancreatic preparations*

The proteolytic activities, assayed according to Anson (Anon, 1966), of the commercial pancreatic preparations Pankipsin and Neopankreatin were 277 $\pm$ 5 U $g^{-1}$ and 28 $\pm$ 0.74 U $g^{-1}$, respectively, i. e., the former preparation obtained by repeated precipitations with $Na_2SO_4$, had a tenfold activity as compared to the other one. This suggests the latter to be a spray- or freeze-dried crude pancreatic extract which might show other enzyme activities (e. g. amylase, lipase) as well.

The trypsin and chymotrypsin activities of Pankipsin and crystalline commercial preparations are given in Table 1.

The data confirm BAEE to be a more sensitive substrate of trypsin activity than DL-BAPA. The ratio of the activity values obtained on the two substrates was 4.3. It can be seen further that the crystalline commercial trypsin preparation is, to some extent, contaminated by chymotrypsin. The ratio of the two activities (assayed on BAEE and BTEE, resp.) in the units applied was found

Table 1

*Trypsin and chymotrypsin activities of pancreatic proteases assayed on specific substrates* (U $g^{-1}$)

| Enzyme preparation | Substrate | | | | | |
|---|---|---|---|---|---|---|
| | BAEE | | DL-BAPA | | BTEE | |
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| Pankipsin | 4.80 | 0.12 | 1.11 | 0.026 | 3.20 | 0.006 |
| Chymotrypsin, crystalline, bovine (Reanal) | 0.027 | 0.001 | — | — | 6.00 | 0.044 |
| Trypsin, crystalline, bovine (Merck) | 7.48 | 0.089 | 1.75 | 0.026 | 0.293 | 0.023 |

BAEE: N-α-benzoyl-L-arginine ethyl ester hydrochloride
DL-BAPA: N-α-benzoyl-DL-arginine-4-nitroanilide hydrochloride
BTEE: N-benzyol-L-tyrosine ethyl ester
U: enzyme activity unit; 1 U: the change in absorbance caused by 1 mg of enzyme protein in 1 min at 255 nm (for BAEE and BTEE) and 410 nm (for DL-BAPA), resp.
$\bar{x}$: mean of 3 parallel determinations
$\pm s$: standard deviation

to be 25.5. The corresponding value for Pankipsin was 1.5. The crystalline commercial chymotrypsin was practically devoid of trypsin activity, the latter could be detected only with the more sensitive BAEE substrate.

### *2.2. Isoelectric points of the enzymes*

The results of IF of the preparation Pankipsin as well as of crystalline bovine trypsin and chymotrypsin are shown in Fig. 1, the respective patterns of the ovine+caprine enzymes separated by affinity chromatography in Fig. 2.

Summarizing the results of a total of five runs, it can be said that the preparation Pankipsin was separated into 5 groups of proteins with the following pI-s: 10.6; 9.5; 6.2–7.2; 4.7–5.3 and approximately 4. The bovine chymotrypsin focused at pI 9.1–9.2, the bovine trypsin at pI 10.8. The respective values for the ovine+caprine enzymes were pI 9.5 and 10.6, somewhat more alkaline and acidic, respectively, than the values found for the bovine enzymes. The resolution capacity of IF could be further improved by modifying the conditions (support: Agarose C, duration of run: 165 min). In this case the crystalline bovine chymotrypsin showed, beside the pI 9.2 fraction, another band of pI 8.4 and the crystalline bovine trypsin showed, beside the pI 10.8 fraction, another band of pI 10.6. The crystalline porcine trypsin of Merck's showed also two main bands: one of pI 10.5 and a second one of pI 10.2, and so did the ovine + caprine trypsin obtained by affinity chromatography. However, α-chymotrypsin had but one fraction of pI 9.1. These results are illustrated in Fig. 3.

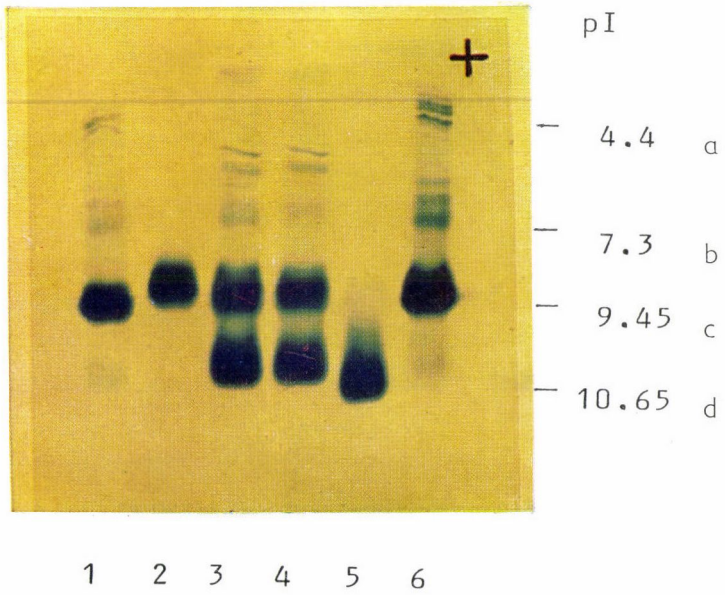


Fig. 1. Results of isoelectric focusing of commercial pancreatic preparations. Experimental conditions: agarose–sorbit thin-layer on plastic support; pH gradient 4–11 established with Servalyt T4-9 and Servalyt T9-11 (Serva, Heidelberg); run performed in flat-bed apparatus of Pharmacia (Uppsala) for 2 h at 50–1000 V under cooling. Amounts applied: 3–15 $mm^3$ of 10–25 g $dm^{-3}$ protein solutions. 1 and 6: standard protein mixture; 2: crystalline bovine chymotrypsin (Reanal, Budapest); 3 and 4: ovine+caprine pancreatic protease Pankipsin (Meat Combinate, Ulan-Bator); 5: crystalline bovine trypsin (Merck, Darmstadt). Composition of standard protein solution and respective pI-s (in brackets): *a:* ferritin (4.4); *b:* horse myoglobin (7.3); *c:* ribonuclease (9.45) and *d:* cytochrome *c* (10.65), the latter from Reanal (Budapest), the other three from Serva (Heidelberg). The pI values of the polymorphous standard enzymes were established according to Serva instructions. This pattern is representative of five runs

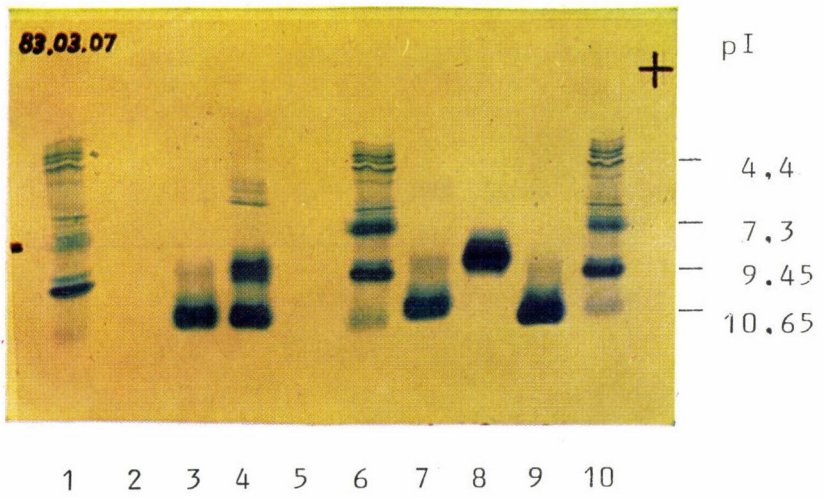


Fig. 2. Results of isoelectric focusing of trypsin and chymotrypsin preparations. For experimental conditions and composition as well as isoelectric points of the standard protein mixture see Fig. 1. 1, 6 and 10: standard protein mixture; 2 and 5: chymotrypsin, ovine+caprine, obtained by affinity chromatography; 3 and 7: trypsin, ovine+caprine, obtained by affinity chromatography; 4: Pankipsin (Meat Combinate, Ulan-Bator); 8: crystalline bovine chymotrypsin (Reanal); 9: crystalline bovine trypsin (Merck)

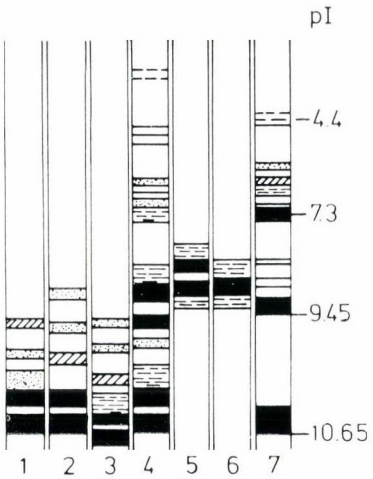


Fig. 3. Results of prolonged isoelectric focusing of commercial pancreatic proteases and purified trypsin and chymotrypsin preparations. Duration of run: $2^3/_4$ h. The rest of experimental conditions is identical to those described in Fig. 1. 1: ovine+caprine trypsin obtained from the commercial preparation Pankipsin; 2: crystalline porcine trypsin (Merck, Darmstadt); 3: crystalline bovine trypsin (Merck, Darmstadt); 4: ovine+caprine pancreatic protease Pankipsin (Meat Combinate, Ulan-Bator); 5: crystalline bovine chymotrypsin (Reanal, Budapest); 6: bovine α-chymotrypsin, 3×crystallized (Sigma, Saint Louis); 7: standard protein mixture

### *2.3. Sodium-dodecyl sulfate–polyacrylamide gel thin-layer electrophoresis*

The results of SDS–PAG thin-layer electrophoresis are shown in Figs. 4 and 5.

The crystalline bovine chymotrypsin and α-chymotrypsin preparations were resolved into several protein fractions. The molecular mass ($M_r$) of the upper band (of lowest mobility) corresponds to about 25 000, this is followed by a faint fraction of $M_r$ 13 600, a distinct zone of $M_r$ 12 500 and three fractions of $M_r$ values below 11 000.

The band of $M_r$ 27 000 in the pattern of the standard protein mixture might be due to the dimer of ribonuclease (HAJÓS & DELINCÉE, 1983). The electrophoretogram of bovine trypsin showed a doublet in the $M_r$-range of 23 000–24 000. In this range porcine trypsin gave a single band of a somewhat lower $M_r$. The electrophoretograms of all the trypsin preparations contained a band of $M_r$ 13 000 and another one of $M_r$ 10 000. Fractions of $M_r$ values below 10 000 could be found as well.

In the commercial preparations Pankipsin and Neopankreatin the slowest moving band can be seen in the $M_r$-range around 54 000. In the range between $M_r$ 25 000 and $M_r$ 54 000 no fraction appeared in the pattern of Pankipsin, while 4 bands showed in that of Neopankreatin: one around 51 000, another one around 40 000 and two around 37 000. The main protein fractions were to

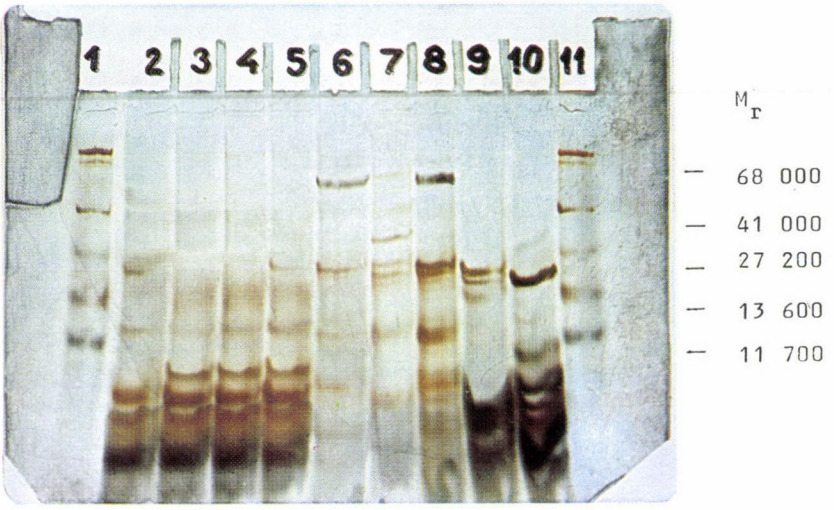


Fig. 4. Results of sodium dodecyl sulfate (SDS) – polyacrylamide gel (PAG) thin-layer electrophoresis of commercial pancreatic proteases as well as of purified commercial trypsin and chymotrypsin preparations. Experimental conditions: 100 g $dm^{-3}$ PAG containing urea. Proteins dissolved in 0.01 mol $dm^{-3}$, pH 7.2 phosphate buffer containing SDS and mercaptoethanol; dilutions of 0.5 g $dm^{-3}$ applied in amounts of 10–15 $mm^3$; electrophoresis carried on for 1 h at 50 V, r. t. and for another 225 min at 100 V, 5 °C; staining, after denaturation with 20% TCA, with $AgNO_3$, according to MERRILL (1982) and HAJÓS & DELINCÉE (1983). Composition of standard solution with molecular masses in brackets: cytotchrome *c* (11 700), ribonuclease (13 600), dimer of ribonuclease (27 200), alcohol dehydrogenase (41 000) and bovine serum albumin (68 000). 1 and 11: standard proteins; 2: crystalline bovine chymotrypsin (Reanal, Budapest); 3: bovine α-chymotrypsin, 3×crystallized (Sigma, St. Louis); 4: crystalline α-chymotrypsin (Calbiochem, Lucerne); 5: chymotrypsin, 3×crystallized (Serva, Heidelberg); 6 and 8: ovine+caprine pancreatic protease Pankipsin (Meat Combinate, Ulan-Bator); 7: pancreatic protease Neopankreatin (Gedeon Richter, Budapest); 9: crystalline bovine trypsin (Merck, Darmstadt); 10: crystalline porcine trypsin (Merck, Darmstad)

be found in the $M_r$-range of 23 000–27 000. This range corresponds to chymotrypsin and trypsin. Fractions in the $M_r$-range of 11 000–13 000 can be seen in the electrophoretograms of both preparations.

The ovine+caprine chymotrypsin fraction gave, in contrast to bovine chymotrypsin, only one very distinct protein zone of $M_r$ around 25 000 and a second very faint band around $M_r$ 12 000.

The ovine+caprine trypsin fraction showed a very broad zone of dark colouration in the $M_r$-range of 23 000–25 000, further, several fractions of $M_r$ values in the $M_r$-range 11 000–13 000 and in the range below these values.

*2.4. Dependence of activity of ovine+caprine trypsin on pH*

The pH-dependence of activity of the trypsin fraction obtained by affinity chromatography of the preparation Pankipsin is shown in Fig. 6.

Both the purified ovine+caprine trypsin and the preparation Pankipsin showed highest activity at pH 8.13, while the bovine trypsin preparation used


Fig. 5. Results of sodium dodecyl sulfate (SDS) – polyacrylamide gel (PAG) thin-layer electrophoresis of ovine+caprine trypsin and chymotrypsin as well as of commercial pancreatic enzyme preparations. Experimental conditions: as in Fig. 4. 1 and 11: standard proteins; 2: crystalline bovine chymotrypsin (Calbiochem, Lucerne); 3: crystalline bovine chymotrypsin (Reanal, Budapest); 4: ovine+caprine chymotrypsin obtained from Pankipsin by affinity chromatography; 5: and 7: ovine+caprine pancreatic protease Pankipsin (Meat Combinate, Ulan-Bator); 6: pancreatic protease Neopankreatin (Gedeon Richter, Budapest); 8: ovine+caprine trypsin obtained from Pankipsin by affinity chromatography; 9: crystalline bovine trypsin (Merck, Darmstadt); 10: crystalline porcine trypsin (Merck, Darmstadt). For standard proteins see Fig. 5

for comparison had its optimum activity in a broader pH-range, between 7.55–8.13. The activity–pH curve of the ovine+caprine enzyme had a sharp maximum: the section between pH 7 and the maximum was steeper than with bovine trypsin. E. g., at pH 7.07 the activity of the former constituted 75% of the maximum value, while for the latter the corresponding value amounted to 95%. As to the descending section of the activity–pH curves, it can be said that the decrease in activity was similar for the ovine + caprine and the bovine enzyme. The descending section was somewhat steeper for the purified ovine + caprine enzyme: this seems to be somewhat less stable in the pH-range above 8.18 than the other two preparations tested.

### *2.5. Dependence of activity of ovine+caprine trypsin on temperature*

The temperature-dependences of ovine+caprine trypsin obtained by affinity chromatography from the pancreatic protease preparation Pankipsin and of Pankipsin itself are shown in Fig. 7.

Both preparations had maximum activity at 50 °C. The maximum was narrower with Pankipsin.

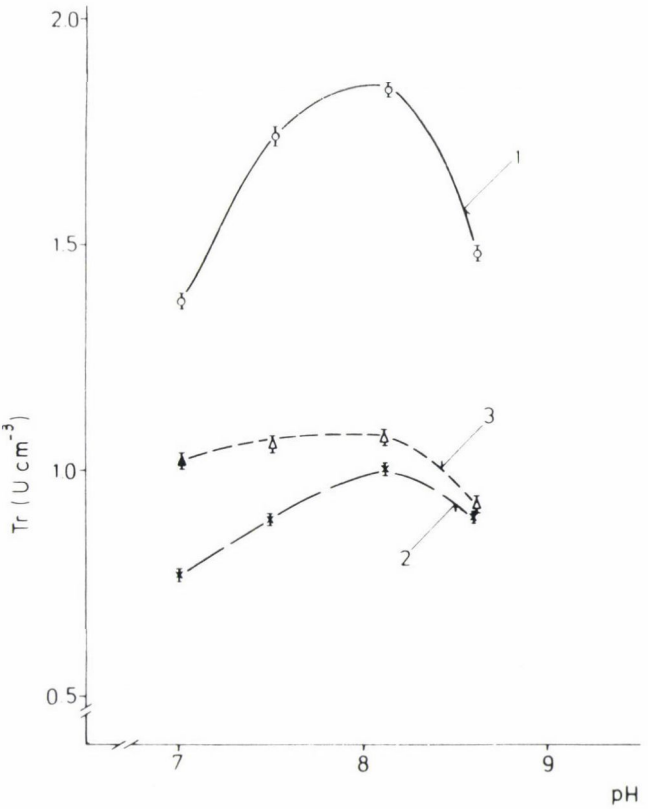


Fig. 6. Dependence of trypsin activity on pH. 1: trypsin separated by affinity chromatography from the ovine+caprine pancreatic protease Pankipsin; 2: Pankipsin (Meat Combinate, Ulan-Bator); 3: crystalline bovine trypsin (Merck, Darmstadt). Activities were assayed on DL-BAPA (N-α-benzoyl-DL-arginine-4-nitroanilide hydrochloride) (ERLANGER et al., 1961)

### *2.6. Heat inactivation of pancreatic proteases*

The results of the heat inactivation experiments carried out with the ovine+caprine pancreatic protease preparation Pankipsin, its trypsin fraction obtained by affinity chromatography and a crystalline bovine trypsin preparation (Merck, Darmstadt) are shown in Fig. 8.

Residual activities measured at pH 8.0 and r. t. after 10-min heat treatments at 60 °C were, as expressed in % of the activity of the untreated sample: 6.5 for Pankipsin, 14 for crystalline bovine trypsin and 43.3 for the ovine + caprine trypsin fraction. After similar treatment at 70 °C the corresponding values were: 0.27, 6.9 and 2.5. These data show Pankipsin to be the least and its trypsin fraction to be the most heat stable.

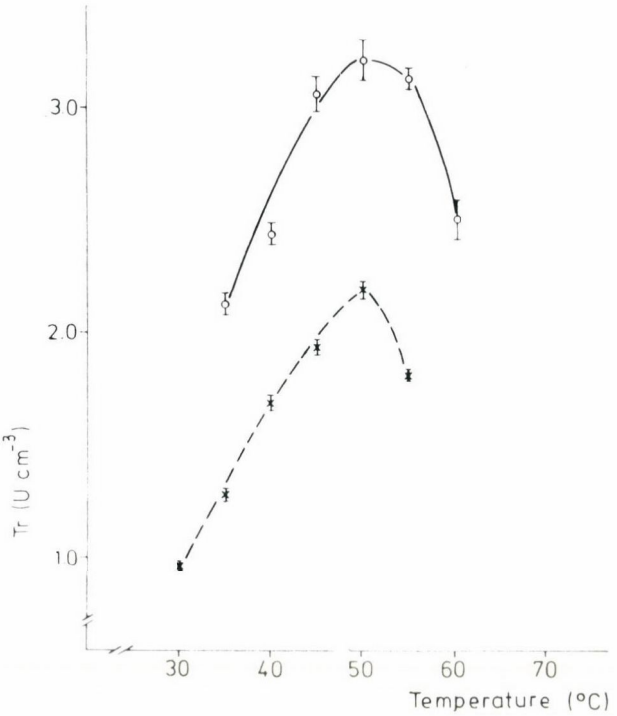


Fig. 7. Dependence of the activities of ovine + caprine proteases on temperature. —: trypsin obtained by affinity chromatography from the commercial preparation Pankipsin (Meat Combinate, Ulan-Bator); - - -: Pankipsin. Activities were assayed on DL-BAPA substrate (see legend to Fig. 6)

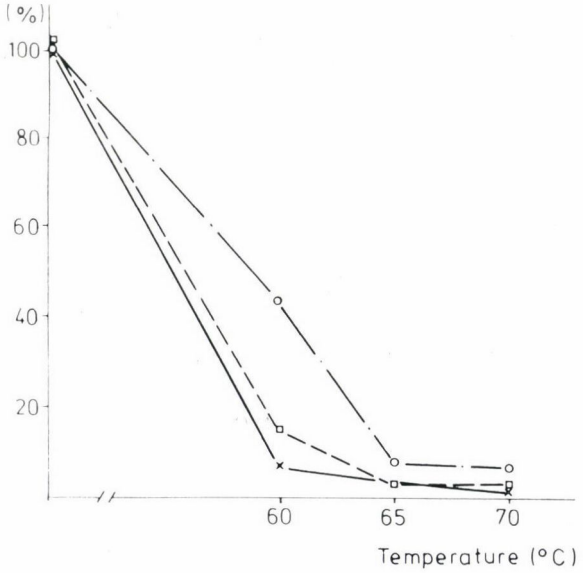


Fig. 8. Heat inactivation of pancreatic proteases. Residence time at the selected temperatures: 10 min. — — —: trypsin obtained by affinity chromatography from the commercial preparation Pankipsin (Meat Combinate, Ulan-Bator); - - - -: crystalline bovine trypsin (Merck, Darmstadt); —: Pankipsin. Residual activities were assayed after heat treatment, at r. t., on DL-BAPA substrate according to a modification of the method of ERLANGER and co-workers (1961) (see para. 1.8. of text) and expressed as % of the value found for the untreated sample

## 3. Conclusions

### *3.1. Isoelectric focusing*

The high-resolution method of IF applied in the present study revealed some fractions in the ovine+caprine pancreatic protease preparation Pankipsin which could be tentatively identified by comparison with pI-data of the literature (RAUEN, 1964) as accompanying enzymes: the band of pI 6.2–7.2 might be carboxypeptidase, that of pI 4.5–5.3 lipase and/or α-amylase, while the pI 4 fraction might indicate the presence of desoxyribonuclease, all of these enzymes occurring in the pancreas. The presence of a zymogen cannot be precluded either. Although no data were found on the pI-s of ovine or caprine chymotrypsinogen, these might give rise to the fractions of pI-s 4.7–5.3 and 6.2–7.2. The pI of bovine trypsinogen B was found to be in the range of 4.5–5.0 and that of porcine trypsinogen A of the value 7.2 (MOSOLOV, 1971).

Trypsin and chymotrypsin obtained from the pancreas of sheep of the Krasnodar region (Soviet Union) were found, as determined by paper electrophoretic mobilities, to have pI-s of 10.6 and 9.0 (PROSKURYAKOV, 1974). Both values are somewhat lower than those obtained for the enzymes from Mongolian sheep and goat pancreas. These differences might arise from differences in general and/or breeding conditions, climate, feed, etc., which might have a bearing on the structures of pancreatic enzymes. The chymotrypsin impurity of the ovine + porcine trypsin preparation (see Part I) is discernible also in the electrophoretic pattern. The crystalline bovine trypsin preparation of Merck shows the same impurity as well (Fig. 2).

The results obtained on modified focusing (Fig. 3) suggest, beside the presence of α-chymotrypsin, in the crystalline bovine chymotrypsin preparation of Reanal an intermediate form absent from the bovine α-chymotrypsin preparation of Sigma. The trypsin doublets in the electrophoretograms of the ovine+caprine as well as the bovine and porcine trypsin preparations might correspond to β- and α-trypsin (SCHROEDER & SHAW, 1968). It can be equally seen that, in contrast to data of the literature, the ovine (+caprine) trypsin is more similar to the corresponding porcine than to the bovine enzyme, at least as far as pI-s are concerned.

### *3.2. Molecular mass estimation*

The main fractions of trypsin of various origins with $M_r$ values in the range of 23 000–24 000 correspond to β-trypsin consisting of a single polypeptide chain. The doublet found in the electrophoretogram of crystalline bovine trypsin might indicate the presence of chymotrypsin as an impurity. This impurity could be equally detected by activity measurements and IF (Table 1 and Fig. 2). The electrophoretic pattern of porcine trypsin does not

show such a doublet. According to MOSOLOV (1971), commercial porcine trypsin preparations are of higher purity than those of bovine origine: by titration of the active site of crystalline trypsin preparations it was established that those of bovine origine contained up to 50% of inactive parts, while in the porcine ones this value reached but 17–25%.

The two bands of $M_r$-s 13 000 and 10 000 each as observed in the patterns of all the trypsin preparations investigated shows these to contain $\alpha$-trypsin which, when treated with mercaptoethanol, splits up into two fractions of $M_r$ 13 000 and $M_r$ 10 000, respectively. PRICE (1976) found, by SDS–PAG electrophoresis, the trypsin preparations of Sigma (St. Louis) and Worthington (Freehold, N. J.) to contain, beside 68–77% $\beta$-trypsin, also $\alpha$-trypsin in which the bond between Lys 131 and Ser 132 had been split (SCHROEDER & SHAW, 1968). This resulted, upon mercaptoethanol addition, in the formation of two peptide chains of $M_r$-s of 13 000 and 10 000, respectively (PRICE, 1976). The same author found also that $\psi$-trypsin, in which the bonds Lys 131–Ser 132 and Lys 176–Asp 177 had been split (SMITH & SHAW, 1969), were degraded, upon the action of mercaptoethanol, to three peptide chains of $M_r$-s 13 000, 4 700 and 5 500, respectively (PRICE, 1976). In the bovine trypsin preparation of Merck's investigated in the present study the main protein band ($M_r$ 23 000–24 000) corresponds to $\beta$-trypsin; the fractions of $M_r$-s 13 000 and 10 000 indicate the presence of $\alpha$-trypsin, while those of $M_r$-s below 10 000 might be due to $\psi$-trypsin and to peptides formed as a result of autolysis. The broad main band ($M_r$ 23 000–25 000) of ovine+caprine trypsin might be due to differences in the molecular masses of the two kinds of animals but also to the slight chymotrypsin contamination (this seems to correspond to a separate band in the electrophoretogram of crystalline bovine trypsin).

In the crystalline chymotrypsin preparations the fractions around $M_r$ 25 000 — present as main band in the electrophoretogram of the ovine + caprine preparation — correspond to $\alpha$-chymotrypsin. The fractions of lower molecular masses might be due to splitting of the latter to the chains A, B and C (MOSOLOV, 1971; ELŐDI, 1980) and/or to autolysis of the enzyme.

In the commercial pancreatic proteases Pankipsin and Neopankreatin, the slowest moving fraction might well correspond to $\alpha$-amylase as this has a $M_r$ of 51 000–54 000 (ELŐDI, 1980). This corroborates our findings related to pI determination (fraction of pI 5.3, see para. 3.1). The 4 bands of Neopankreatin in the $M_r$-range 37 000–51 000 might correspond to lipase ($M_r$ 45 000–50 000) and carboxypeptidase ($M_r$ 35 000) (MOSOLOV, 1971). These fractions are absent from the electrophoretogram of Pankipsin. This again bears out our assumption that Neopankreatin is a less purified preparation than Pankipsin (see para. 2.1). The fractions of $M_r$-s around 11 000 and 13 000 as found in both preparations might correspond to fragments of $\alpha$- and $\psi$-trypsin, but might also indicate the presence of ribonuclease ($M_r$ 13 700, pI 9.45) (see para. 2.1).

*3.3. Dependence of trypsin activity on pH and temperature*

In agreement with the results presented here, BUCK and co-workers (1962) found the pH-optima of ovine, bovine and porcine trypsins on BAEE substrate likewise in the range of pH 8.0–8.2. Thus, neither the origin, nor the substrate seems to affect the pH-optimum of trypsin. The dependence of activity on pH as measured in a broader range seems more suited to differentiate between trypsins of various origin and purity as was shown in Fig. 6.

When comparing proteolytic activities of bovine, porcine and ovine trypsin on 20 g $dm^{-3}$ casein substrate in the temperature range of 35–65 °C, the same authors found, in the above order, activity maxima at 45 °C, 50–55 °C and 45–50 °C, respectively, whereby the activity of the enzyme from sheep as measured at 35 °C was half that obtained at 50 °C. The corresponding values for Pankipsin and its trypsin fraction were 58% and 66%. These data show

— bovine trypsin to be the least and porcine trypsin to be the most heat stable of the three enzymes,

— ovine trypsin to have a temperature optimum broader than that of the bovine and somewhat narrower than that of the porcine enzyme and

— pancreas protease from Mongolian sheep+goat to have the broadest temperature optimum of all the preparations compared.

*3.4. Heat inactivation of trypsin*

The higher heat resistance of ovine+caprine trypsin obtained by affinity chromatography from Pankipsin as compared to the latter leads to the conclusion that out of the main components of Pankipsin, i. e., trypsin, chymotrypsin and the proteins not binding to the affinity column (cf. Part I), the first one is the most heat stable. The results of the heat inactivation trials carried out with Pankipsin, its trypsin fraction and the crystalline bovine preparation of Merck's are in keeping with our findings and data from the literature related to the temperature dependence of activity of these enzymes (see preceding para.).

Summing up, it can be said that the proteolytic enzymes of the pancreas of the Mongolian sheep+goat differ, in many respects, not only from the corresponding enzymes of bovine or porcine origin, but also from the ovine pancreatic enzymes of other geographic regions. These differences in protein character might, at least partly, explain the failure of separating trypsin and chymotrypsin of the Mongolian sheep + goat by ion exchange chromatography, a method found fit to separate these enzymes from other sources (cf. Part I).

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# EFFECTS OF SOME ADDITIVES AND PROCESSES ON THE CHARACTERISTICS OF AGGLOMERATED AND GRANULATED SPRAY-DRIED ROSELLE POWDER

A. H. EL TINAY and I. A. ISMAIL

Department of Biochemistry, Faculty of Agriculture, Shambat, Sudan

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The water extract of Roselle (known in Sudan as Karkade) calyces was used in the preparation of spray-dried Karkade powder. A mixture of 10% spray-dried powder and 90% sucrose was used in the preparation of granules of different particle sizes and agglomerates. The incorporation of sugar was successful in producing less hygroscopic granules and agglomerates having higher bulk densities, better reconstitution characteristics and good keeping qualities as far as hygroscopicity is concerned, compared to the spray-dried powder. The agglomerates being of best reconstitution characteristics were further improved by vacuum drying (26.7 kPa), moistening to low initial moisture level (6%) and drying at low temperature (45 °C) for 30 min. Agglomerates treated with 0.45% of very fine tricalcium phosphate (TCP) powder were less hygroscopic and showed slight caking at a comparatively high relative humidity (43%).

**Keywords:** Roselle (Karkade), spray-drying, vacuum drying, hygroscopicity

Roselle *(Hibiscus sabdariffa)* is known in Sudan as Karkade. The sweetened water extract of Karkade calyces is a popular beverage in Sudan. Water extracts of Karkade had been used for producing a spray-dried Karkade powder. The spray-dried powder being produced has poor reconstitution characters and is highly hygroscopic which results in quick caking. In formulating products to enhance their instant properties, some products require additives either because they are otherwise difficult to agglomerate, or because agglomeration alone was not sufficient to achieve instant properties. Sugar is a common ingredient of enhancing the instant properties of many beverage products (JENSEN, 1975). Insoluble phosphates are very commonly used as anti-caking agents for hygroscopic powders. ELLINGER (1972) found that the addition of tricalcium phosphate to a powdered beverage mix beneficially influenced not only the flow properties but also the particle size distribution of the dry mix.

The objective of this work is to improve the reconstitution characters of spray-dried Karkade powder and to increase the shelf-life as far as hygroscopicity is concerned by the use of additives, and increase in particle size under controlled conditions.

## 1. Materials and methods

### *1.1. Sample preparation*

Dry Karkade calyces, El Rahad variety, was purchased from Khartoum local market.

Spray-dried Karkade powder was prepared by soaking the dry crushed calyces (5 mm particle size) in distilled water at ambient temperature (35 °C), in the ratio 1:9 for 3 h. Extraction was carried out batchwise without agitating the system (JACKSON & BASHIR, 1968a). Tap water was slightly alkaline and influenced the colour and pH of the spray-dried powder; hence distilled water was being used in extraction trays (JACKSON & BASHIR, 1968b). After filtration the extract (7% total soluble solids) was spray-dried using Anhydrospray-drier at an inlet air temperature of 240–250 °C and an outlet air temperature of 90–100 °C and compressed air at 147.1 kPa pressure (BASHIR, 1969).

### *1.2. Additives*

Sucrose of commercial grade was milled and passed through 150 μm mesh sieve. Tricalcium phosphate (TCP) of technical grade was milled to a very fine powder and passed through 74 μm mesh sieve.

### *1.3. Granulation of spray-dried Karkade powder*

To fix the ratio of Karkade to sucrose powder serial proportions of sucrose were used, 10%, 20% 30% etc.; however, it was found that lower sucrose–Karkade powder ratios were very sticky and difficult to granulate. The 1:9 ratio of Karkade to sucrose resulted in an excellent mixture which was easy to formulate and gave high percentage of granulation.

A granulation process was carried out by mixing Karkade powder and sucrose, in the ratio of 1:9, by means of a mortar and pistil. The mixture was then passed through 150 μm mesh sieve before adding water by a hand sprayer. The moisture content of the mixture was maintained at 13%. Granules were prepared by pressing the wet mixture against a mesh and dried in an air oven at 45–50 °C for 2 h. Granules of 1.4, 1.0 and 0.74 mm mesh sizes were separated.

### *1.4. Agglomeration of spray-dried Karkade powder*

*1.4.1. General process description.* The proportion of 1:9 Karkade powder to sucrose powder was also used in the preparation of agglomerates. Agglomerates were produced by manually agitating the moist mixture in a mortar using a pistil. The mixture was wetted by a hand sprayer. The resulting agglomerate was spread on a stainless steel tray to 0.5 cm thickness and then dried. The dry

agglomerate was passed through number 24 mesh sieve (0.7 mm mesh size) to obtain uniform size.

*1.4.2. Process variables.* The following experimental variations were applied for agglomerates prepared as described above.

*1.4.2.1. Atmospheric air drying.* This was carried out using an air oven (Elektrohelios, Sweden) at 45–50 °C for 1 h.

*1.4.2.2. Vacuum air drying.* Agglomerates were dried at 79.9, 53.3 and 26.7 kPa in a Karl Kolb type cylindrical vacuum oven (W. C. Heraeus Hanau, Frankfurt/M, FRG).

*1.4.2.3. Variation of the initial moisture content.* The initial moisture content of the agglomerate before drying was maintained by estimating the starting moisture content of the powder mixture described under para. 1.4.1. and then complementing to the desired level by wetting. Levels used were 6, 8, 10 and 12%.

*1.4.2.4. Variation of the drying temperature.* Drying temperatures of 40, 45, 50, 55 and 60 °C were applied.

*1.4.2.5. Construction of the drying curve.* A drying curve was constructed for a 6% moisture content sample at 45 °C temperature and 26.7 kPa pressure. Moisture content was determined in batches taken at 5 min time intervals. A drying characteristics curve was constructed.

### *1.5. Tricalcium phosphate as an anti-caking agent*

Tricalcium phosphate was added to Karkade powder–sucrose mixture in the following percentages: 0.15, 0.30, 0.45 and 0.60. Agglomeration was carried out as described earlier. An agglomerate of 6% moisture content was dried at 45 °C temperature and 26.7 kPa pressure for 30 min.

### *1.6. Moisture determination*

Moisture content was determined by the accepted method described in the AOAC (1970).

### *1.7. Particle size*

Particle size was determined using an optical microscope equipped with a calibrated micrometer eye-piece. Five hundred particles were measured in each case to assure statistically significant results.

### *1.8. Bulk density*

A weighed sample was transferred to a 100 $cm^3$ graduated measuring cylinder and mounted on a screen vibrator. After vibrating for 5 min, the volume occupied was recorded and the results were expressed in g per $cm^3$ unit.

*1.9. Flowability*

The flowability of the material was determined by finding the angle of repose (KAREEM, 1973).

*1.10. Wettability and sinkability*

The wettability and sinkability of powders are difficult to separate and it is possible to associate them in one test.

This test involves spreading on the surface of a given volume of water of a definite weight of the powder and recording the time which elapses between the spreading of the powder and the disappearance of the last particles from the surface of the water. The time taken for the powder to sink completely was also determined (KAREEM, 1973).

*1.11. Dispersibility*

The dispersibility of the powder material was determined by taking 2 g of the powder which were added to 100 $cm^3$ of distilled water at 20 °C temperature and agitated with a magnetic stirrer at 1000 r. p. m. After 5 s a supernatant of the dispersion was withdrawn by means of a syringe. The sample was centrifuged at 1000 r. p. m. for 3 min and the optical density of the supernatant was measured at 530 $\mu$m. Dispersibility was expressed in terms of optical density units (KAREEM, 1973).

*1.12. Solubility*

The solubility of the samples was determined by adding 10 g of the material to 250 $cm^3$ distilled water at 20 °C temperature. The mixture was immediately stirred using a mechanical stirrer at 1000 r. p. m. and observing the time for the material to dissolve completely.

*1.13. Hygroscopicity*

Ten g of the material was evenly spread in an aluminium dish. The sample was then placed in a constant temperature chamber at 26.7 °C and 52% relative humidity. The gain in weight due to moisture absorption was recorded at 10 min intervals. The hygroscopicity curve was constructed as described by GRIFFIN and LYNCH (1972).

*1.14. Caking test*

The method described by FALICIOTTI (1972) was used to conduct the stability of the material under test at different equilibrium moisture contents, with respect to caking. The test was carried out at different equilibrium relative

humidities. Weighed samples were placed in desiccators, containing saturated salt solutions. Each saturated salt solution had a predetermined relative humidity. The desiccators with their respective samples were placed in a constant temperature chamber at 27.7 °C and 52% relative humidity for 10 days and caking was observed.

*1.15. Sensory quality*

This was assessed in duplicate samples by 10 panelists for colour and flavour. Samples were scored on a scale of 10 points, highest marks indicating better quality. An average score was then calculated for each sample and the data analysed statistically.

## 2. Results

In Table 1 the chemical composition of the dry whole calyces, the spray-dried powder and the product treated with 0.45% tricalcium phosphate on dry weight basis, is shown.

Results of physical and organoleptic analyses of spray-dried Karkade powder, agglomerated powder and granulated powder are shown in Table 2. The spray-dried Karkade powder has the smallest particle size (8.5 $\mu$m) followed by the agglomerated sample and the granulated samples have the largest particle sizes. The particle sizes of the granulated samples ranged from 1000 to 1480 $\mu$m. The bulk density of agglomerated Karkade powders decreased with increasing particle size, but the spray-dried Karkade powder has the lowest bulk density, although it has the smallest particle size. The flowability angles

Table 1

*Composition and pH value of dry whole calyces, spray-dried powder and the product with 0.45% tricalcium phosphate, on dry weight basis*

| Component | Dry whole calyces | | Spray-dried powder | | Product treated with 0.45% TCP | |
|---|---|---|---|---|---|---|
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| Moisture content (%) | 12.60 | 0.178 | 3.0 | 0.163 | 0.92 | 0.077 |
| Crude protein (%) | 9.44 | 0.114 | 13.43 | 0.05 | 1.30 | 0.077 |
| Crude fibre (%) | 14.60 | 0.181 | nil | | nil | |
| Reducing sugars (%) | 3.43 | 0.136 | 4.40 | 0.114 | 0.89 | 0.054 |
| Nonreducing sugars (%) | 3.13 | 0.037 | 3.06 | 0.127 | 89.40 | 0.620 |
| Total anthocyanins (%) | 1.40 | 0.016 | 1.53 | 0.040 | 0.148 | 0.013 |
| Ascorbic acid (mg per 100 g) | 13.46 | 0.866 | 16.50 | 0.473 | 1.23 | 0.073 |
| Titratable acids (%) | 20.90 | 0.216 | 34.87 | 0.249 | 3.47 | 0.057 |
| pH value | 2.70 | 0.008 | 2.65 | 0.014 | 2.60 | 0.016 |

Table 2

*Physical and sensory analysis of Karkade powder,*

| Sample | Moisture (%) | | Particle size ($\mu$m) | | Bulk density (g cm$^{-3}$) | | Flowability (degrees) | |
|---|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| Spray-dried Karkade | 3.0 | 0.25 | 8.5 | 0.54 | 0.50 | 0.20$^{c}$ | 54.23 | 0.027$^{a}$ |
| Granulated samples: | | | | | | | | |
| 1.4 mm mesh | 1.4 | 0.05 | 1480 | 35.6 | 0.57 | 0.36$^{b}$ | 46.13 | 0.212$^{ab}$ |
| 1.0 mm mesh | 1.3 | 0.03 | 1130 | 58.9 | 0.63 | 0.025$^{a}$ | 45.00 | 0.341$^{ab}$ |
| 0.74 mm mesh | 1.3 | 0.04 | 1000 | 147.7 | 0.66 | 0.080$^{a}$ | 42.25 | 0.340$^{ab}$ |
| Agglomerated sample | 1.2 | 0.12 | 136 | 4.4 | 0.69 | 0.075$^{a}$ | 32.65 | 6.360$^{b}$ |

In a column sharing the same letter are not significantly different

of the granulates and agglomerates increased with increasing particle size except for the spray-dried Karkade powder which has the highest angle despite of having the smallest particle size.

The wettability (sinkability) and dispersibility values decreased with increasing particle size (Table 2). The solubility of spray-dried Karkade powder reflects the time of wettability and sinkability of the same powder but it is not true for the dispersibility values. The solubility of agglomerated and granulated powders increased proportionally to the dispersibility rate (Table 2). The solubility is inversely proportional to the rate of wetting and sinking.

Statistical analysis of organoleptic scores for colour and flavour of the spray-dried Karkade powder, agglomerated sample and granulated sample, indicated a significant difference between the scores of the three granulated samples and the spray-dried sample. There was no significant difference between the scores of the agglomerated sample and the spray-dried powder (Table 2). Consequently, they were considered to be of comparable quality.

Figure 1 shows the rate of moisture absorbtion by the spray-dried powder, the 3 granulated samples and the agglomerate. It is evident that the spray-dried powder absorbed moisture at an accelerated rate compared to the other samples. The granule with the largest particle size (1480 $\mu$m) absorbed the least moisture while the absorption rate increased slowly with decreasing granule particle size. The agglomerate absorbed moisture at a high rate in the first 30 min and thereafter at a slower rate.

*granulated samples and agglomerated sample*

| Wettability / Sinkability (s) | Dispersibility ($OD_{530}$) | | Solubility (s) | | Score of sensory test | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Colour | | Flavour | |
| | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| 240 / 242±3.3 | 0.468 | 0.009[a] | 110 | 2.5[a] | 8.0 | 0.31[a] | 7.5 | 0.13[a] |
| 0 / 2±0.41 | 0.148 | 0.004[b] | 90 | 2.4[ab] | 6.2 | 0.50[b] | 6.2 | 0.04[b] |
| 0 / 4±0.41 | 0.159 | 0.002[ab] | 75 | 1.6[ab] | 6.0 | 0.15[b] | 6.0 | 0.05[b] |
| 0.5 / 6±0.82 | 0.160 | 0.001[ab] | 60 | 2.2[ab] | 6.2 | 0.50[b] | 6.1 | 0.05[b] |
| 1 / 10±0.82 | 0.228 | 0.003[ab] | 25 | 0.82[b] | 7.6 | 1.04[a] | 7.0 | 0.27[a] |

($P < 0.05$) as determined by Duncan's multiple range test. Number of parallels is 3

Table 3 shows the effect of different process conditions on the reconstitutability of agglomerates. Results show an increased wettability and sinkability, better solubility and an improved dispersibility with the decrease of air pressure.

A similar result is observed in decreasing the initial moisture content of the agglomerate, and also in decreasing the drying temperature. The agglomerate of 6% initial moisture content dried at 26.7 kPa pressure and 40 °C temperature had the best reconstitution characteristics; yet 40 °C was not considered to be appropriate for drying because of the high moisture content of the product. The drying time curve of the agglomerate with 6% initial moisture content, dried at 26.7 kPa pressure and 45 °C temperature, is given in Fig. 2. Drying was rapid during the first 20 min. Nearly half of the original moisture content (3%) was removed after 5 min. The agglomerate reached a moisture content of 1.0–0.8% after drying for 30 min and the moisture content of the agglomerate remained practically unchanged after that drying period.

The wettability and sinkability of all the samples treated with tricalcium phosphate (TCP) as an anticaking agent were relatively the same (Table 4). Dispersibility and solubility were slightly lower. This was probably due to the poor solubility of TCP in water (STECHER et al., 1960). Samples treated with TCP absorbed moisture at a slower rate than the control sample (Fig. 3). At equilibrium, the spray-dried powder was caked at 23% relative humidity and the control agglomerate at 33% relative humidity. The 0.45% TCP was successful in keeping the free flowing properties at 33% relative humidity and at 43%

Table 3

*Effect of different process variables on the reconstitution characteristics of agglomerates*

| Process parameters | Moisture content (%) | | Wettability / Sinkability (s) | Solubility (s) | | Dispersibility ($OD_{630}$) | |
|---|---|---|---|---|---|---|---|
| | $\bar{x}$ | $\pm s$ | | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| *Pressure* | | | | | | | |
| Atmospheric | 1.17 | 0.13 | $\frac{1}{10 \pm 0.82}$ | 25 | $0.82^{a}$ | 0.224 | $0.008^{a}$ |
| 79.9 kPa | 0.95 | 0.08 | $\frac{0}{9 \pm 0.41}$ | 23 | $0.41^{a}$ | 0.235 | 0.004 |
| 53.3 kPa | 0.90 | 0.06 | $\frac{0}{8 \pm 0.41}$ | 21.5 | $0.41^{a}$ | 0.247 | $0.006^{a}$ |
| 26.7 kPa | 0.90 | 0.0098 | $\frac{0}{8 \pm 0.41}$ | 17 | $0.41^{a}$ | 0.260 | $0.008^{a}$ |
| *Initial moisture* | | | | | | | |
| 12% | 0.95 | 0.08 | $\frac{0}{8 \pm 0.16}$ | 22 | $0.57^{a}$ | 0.235 | $0.011^{a}$ |
| 10% | 0.92 | 0.06 | $\frac{0}{7.5 \pm 0.22}$ | 20 | $0.08^{a}$ | 0.245 | $0.009^{a}$ |
| 8% | 0.85 | 0.04 | $\frac{0}{7.3 \pm 0.22}$ | 18 | $0.40^{a}$ | 0.260 | $0.085^{a}$ |
| 6% | 0.80 | 0.03 | $\frac{0}{7 \pm 0.25}$ | 17 | $0.14^{a}$ | 0.270 | $0.005^{a}$ |
| *Drying temperature* | | | | | | | |
| 60 °C | 0.68 | 0.04 | $\frac{0}{8.5 \pm 0.14}$ | 23 | $0.36^{a}$ | 0.185 | $0.008^{b}$ |
| 55 °C | 0.75 | 0.05 | $\frac{0}{8.2 \pm 0.16}$ | 21 | $0.73^{ab}$ | 0.215 | $0.011^{ab}$ |
| 50 °C | 0.85 | 0.05 | $\frac{0}{7.9 \pm 0.16}$ | 18 | $0.06^{ab}$ | 0.245 | $0.018^{ab}$ |
| 45 °C | 9.0 | 0.08 | $\frac{0}{7.3 \pm 0.16}$ | 16 | $1.33^{ab}$ | 0.269 | $0.012^{ab}$ |
| 40 °C | 1.4 | 0.25 | $\frac{0}{7 \pm 0.50}$ | 15 | $0.11^{b}$ | 0.275 | $0.012^{a}$ |

relative humidity caked only slightly. The 0.6% sample was almost free flowing at 43% relative humidity.

Statistical analysis of sensory scores for colour and flavour gave no significant difference between different treatments compared to the control sample.

## 3. Conclusions

The treatment of spray-dried Karkade powder to form granules and agglomerates resulted in improvement of the physical properties of these products (Table 2). The increase in bulk density of granulated samples and agglomerate, was mainly due to the addition of sucrose. With increasing particle size bulk density decreased except for the spray-dried powder. The decrease of bulk density with increase of particle size might be due to the presence of large interspaces between the particles and hence a larger volume being occupied (COULTER & JENNES, 1964).

It was reported that the finer the powder grain the more easily it will go into solution up to about 75 $\mu$m in diameter beyond which greater finesse was undersirable (COULTER & JENNES, 1964). The particles of such powder dissolve on the outside of little lumps and form a paste which protects the other fine particles within, and renders solution less readily accomplished than would be the case with a slightly coarser grained powder, one which will allow the water to penetrate the mass. This explains the behaviour of spray-dried Karkade powder with respect to its physical characters (Table 2).

The comparative stability of the granules and agglomerates as expressed by the hygroscopicity curves, is mainly due to the inclusion of sugar which is very stable in the granular form under normal atmospheric conditions. The increase in particle size also adds to stability since the ratio of surface area to the particle weight is reduced (GREGG & SING, 1967).

Agglomerates, when produced under vacuum drying, resulted in particles of low moisture content. The decrease in moisture content was probably due to the rapid evaporation under vacuum drying. Improvement of the reconstitution properties at low pressures (Table 3) may be attributed to the somewhat porous nature of the particles. According to VAN ARSDEL (1963), the porous condition of vacuum dried samples is very advantageous in promoting rapid rehydration when the product is used.

Agglomerates of initial low moisture levels had better reconstitution characteristics than those containing higher initial moisture levels. Six percent initial moisture level was found to be optimum (Table 3).

Low drying temperatures (40–45 °C) gave products of better reconstitution properties. However, low moisture contents are obtained at higher temperatures of drying. High drying temperatures (55° and 60 °C) seemed to have caused stronger association of individual particles in aggregates, resulting in lower dispersibility and solubility values. High vacuum drying temperatures should be avoided during processing of Karkade agglomerates.

The drying time curve (Fig. 2) shows that large amounts of moisture were removed in the first 20 min. The optimum vacuum drying time was found to be 30 min.

Table 4

*Reconstitutability, caking properties and sensory test of*

| Sample | Wettability / Sinkability (s) | Solubility (s) | | Dispersibility ($OD_{530}$) | |
|---|---|---|---|---|---|
| | | $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ |
| Control | $\frac{0}{7.5 \pm 0.41}$ | 16 | 0.82[b] | 0.280 | 0.022[a] |
| TCP level | | | | | |
| 0.15% | $\frac{0}{7.5 \pm 0.41}$ | 16 | 0.16[b] | 0.280 | 0.011[a] |
| 0.30% | $\frac{0}{8.0 \pm 0.82}$ | 17 | 1.08[ab] | 0.270 | 0.137[ab] |
| 0.45% | $\frac{0}{8.0 \pm 0.41}$ | 18 | 1.08[a] | 0.270 | 0.012[ab] |
| 0.60% | $\frac{0}{8.0 \pm 0.70}$ | 18 | 0.41[a] | 0.260 | 0.020[b] |
| Spray-dried powder | | | | | |

For legends see Table 2

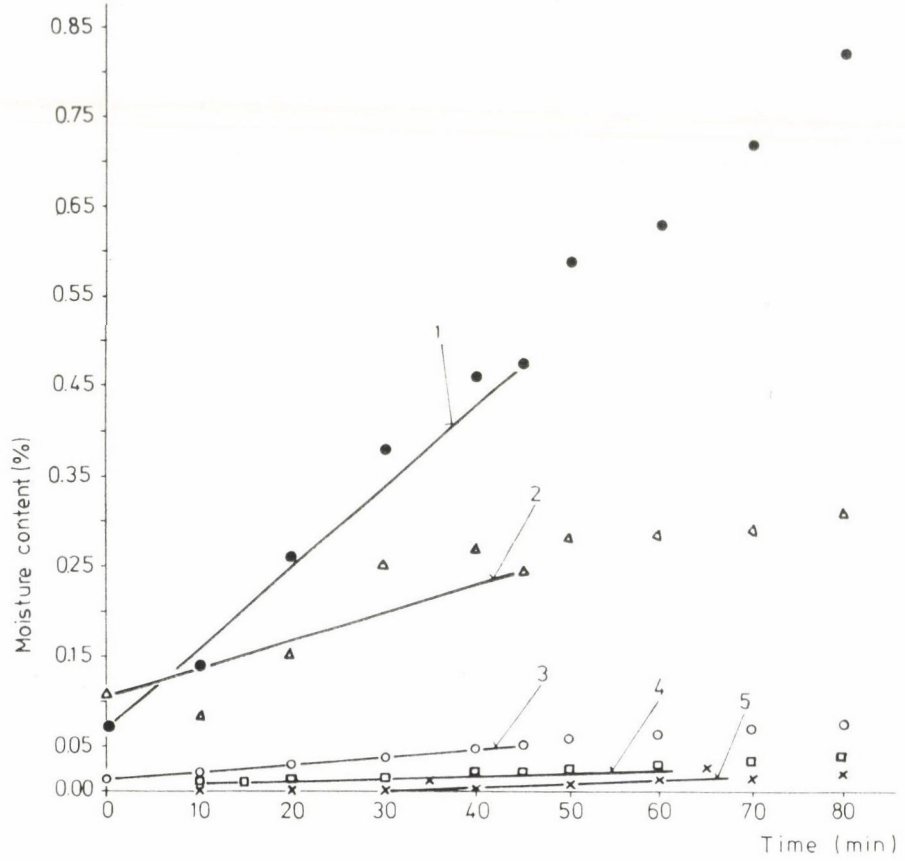


Fig. 1. Moisture absorption of spray-dried Karkade powder, 3 granulated samples and the agglomerate at 52% relative humidity and at 26.7 °C temperature. 1: Spray-dried Karkade powder, $y = 0.07+0.0009x \pm 0.0004$; 2: agglomerated sample, $y = 0.109+ +0.003x \pm 0.0007$; 3: sample granulated (0.74 mm mesh), $y = 0.15+0.0008x \pm 0.0001$; 4: sample granulated (1.0 mm mesh), $y = 0.004+0.0004x \pm 0.00003$; 5: sample granulated (1.4 mm mesh), $y = 0.007+0.0003x \pm 0.00004$. (Correlations: 99%)

*agglomerates treated with tricalcium phosphate (TCP)*

| Scores of sensory test | | | | Caking test (R.H.) | | | |
|---|---|---|---|---|---|---|---|
| Colour | | Flavour | | | | | |
| $\bar{x}$ | $\pm s$ | $\bar{x}$ | $\pm s$ | 23% | 33% | 43% | 52% |
| 7.8 | 0.06[a] | 7.4 | 0.07[a] | Free flowing | Slightly caked | Caked | Caked |
| 7.7 | 0.24[a] | 7.3 | 0.76[ab] | Free flowing | Free flowing | Caked | Caked |
| 7.6 | 0.15[a] | 7.2 | 1.02[ab] | Free flowing | Free flowing | Caked | Caked |
| 7.3 | 1.00[a] | 7.2 | 0.84[ab] | Free flowing | Free flowing | Slightly caked | Caked |
| 7.3 | 0.88[a] | 7.0 | 0.22[b] | Free flowing | Free flowing | Slightly caked | Caked |
| | | | | Caked | Caked | Severly caked | Almost liquified |

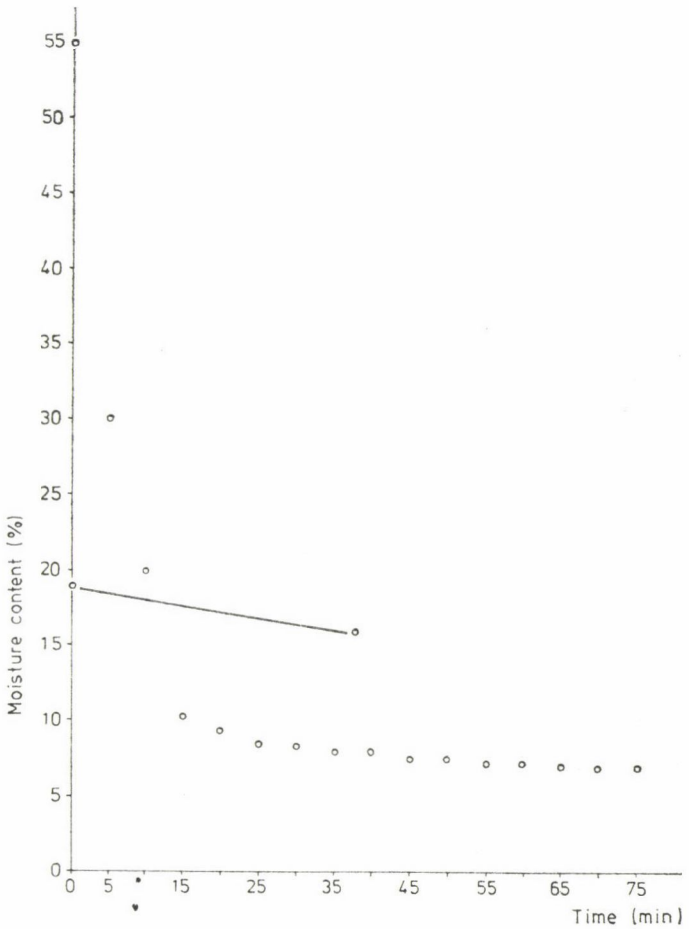


Fig. 2. Drying curve of agglomerate of 6% moisture content at 45 °C temperature and 26.7 kPa pressure. $y = 19.89 - 0.08x \pm 0.04$. (Correlation: 99%)

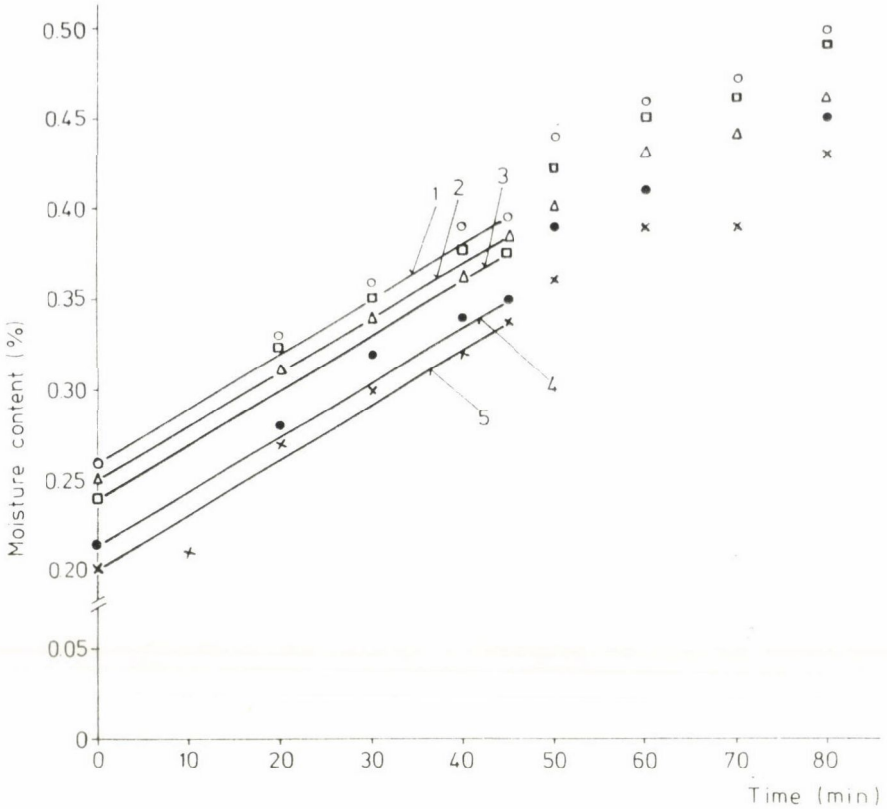


Fig. 3. Moisture absorption of agglomerates treated with tricalcium phosphate at 52% relative humidity and at 26.7 °C temperature. 1: Sample with 0% TCP, $y = 0.26 + 0.003x \pm 0.00024$; 2: sample with 0.3% TCP, $y = 0.25 + 0.003x \pm 0.0002$; 3: sample with 0.15% TCP, $y = 0.24 + 0.003x \pm 0.0007$; 4: sample with 0.45% TCP, $y = 0.215 + 0.003x \pm 0.0002$; 5: sample with 0.6% TCP, $y = 0.203 + 0.003x \pm 0.00023$. (Correlations: 99%)

Samples treated with TCP as an anticaking agent showed some improvement on the caking properties of agglomerates. The TCP was found as being tasteless and colourless, and hence it did not interfere with the flavour of the product. There was no significant difference in the organoleptic scores of the treated samples compared to the control sample (Table 4). The 0.45% TCP was sufficient in improving the free flowing properties at 33% relative humidity and to some extent at 43% relative humidity. The 0.45% level was therefore considered to be satisfactory. CRISTOFARO and WUHRMAN (1972) used 0.39% TCP as an anticaking agent in producing a free flowing dry synthesised orange agglomerate.

From the above discussion we can conclude that an agglomerate of comparatively good reconstitution properties, in terms of wettability and

sinkability compared to the spray-dried Karkade powder could be obtained at 6% initial moisture content, 26.7 kPa drying pressure, 45 °C drying temperature, and 30 min drying time.

The moisture content of the final product was approximately 1%. Further studies should be directed to investigate the safety of this moisture level during storage and in particular its relationship to the thermal degradation of Karkade anthocyanins, particularly because the product contains a large amount of sucrose which accelerates the rate of pigment degradation (TINSLEY & BOCKIAN, 1960).

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# BOOK REVIEW

## Antimicrobials in foods

(Food science, Vol. 10.)

A. L. BRANEN and P. M. DAVIDSON (Eds.)

Marcel Dekker Inc., New York, 1983, 419 pages

An important kind of food additives are the antimicrobial agents. In addition to physical methods of conservation the use of chemical compounds is very important, too. The utilization of antimicrobials has been and is still highly disputable. The question is worth reexamination in view of new discoveries. Biochemical, biophysical, microbiological, food toxicological knowledge, new analytical methods of food control, new food regulations indicate the revision of certain earlier opinions.

The authors discuss related problems in 13 chapters, on 419 pages with 61 tables and a great number of references. Of the number of antimicrobial additives the following are treated: sodium benzoate, benzoic acid, phenolic compounds, organic acids, medium chain fatty acids and esters, sorbates, sulfur dioxide and sulfites, nitrite. The following are discussed as sanitizers: halogens and surface active agents, dimethyl dicarbonate and diethyl dicarbonate, nisin and other inhibitory substances from lactic acid bacteria, antibiotic residues and their significance and natural occurrence and miscellaneous food antimicrobials.

The spectre and mechanism of effect, practical application, toxicology, detection of the compounds, quantitative determination and regulations of their use are reviewed. Useful information on the chemical and physical properties of the antimicrobial agents and on factors affecting them and on the necessary dosages in relation to individual microorganisms are available in the book. Data on the toxicology and legislation related to the permissible dosages in different countries are of great importance.

The mass of information contained in the book is useful for food chemists, toxicologists and microbiologists and can be successfully applied in post graduate training.

I. KISS

*Acta Alimentaria, Vol. 14 (3), pp. 299—301 (1985)*

# IN MEMORIAM

The Editorial Board of Acta Alimentaria deeply regrets to announce the death on 22 April, 1985 of Doctor E. Almási, one of its members since the foundation of the periodical, Director of the Institute of Food Technology, Professor, University of Horticulture. Professor Almási was a well known authority on food science and particularly on refrigeration.

Elemér Almási was born in Bükkösd (County Baranya, Hungary) on 8 December 1919. He took his final examination at the secondary school with honour in Pécs in 1938. In the same year he was accepted as a student in the Faculty of Chemical Engineering, Budapest Technical University. He graduated in 1943 with distinction. He started his working career in the Institute for Chemical Technology of the Technical University, his next job was in the Institute of Fermentation and from there he went to work in the Nagyatád Canning Factory. Between 1943 and 1948 he did his military service.

On returning to Budapest he was engaged as Chief of Laboratory at the Mirelite Deep-freezing Enterprise. In 1950 he became a member of the Research Institute for the Canning, Meat and Refrigeration Industries. In 1959 he was appointed Head of the Refrigeration Department of the Institute.

In December 1967 he was appointed Professor of Food Technology and Microbiology at the University of Horticulture. Therewith he started his teaching career.

During the first two years he lectured on Food Technology at the Cultivation Department of the University. When in 1970 the Faculty for Food

Preservation was organized he started his lectures on Refrigeration Technology and lead the practices in the same subject, thereby breaking new ground. He also took part in the organization of education at the Faculty for Food Preservation and in the development of the first curriculum. The difficulty of this task was increased by the fact that a new educational system, two level teaching was introduced, which was at the time a novelty in Hungary. Between 1971 and 1974 he was deputy rector of the University of Horticulture, responsible for foreign matters. When the Institute of Food Technology was founded he was nominated first director and was asked to direct the Department of Food and Refrigeration Technology. Beside instructing at graduate level he acted as a tutor to postgraduate students, as well. He has been engaged in research activities, mainly in the field of cold storage and quick-freezing for a long time. While working at the Research Institute for the Canning, Meat and Refrigeration Industries he studied the possibilities of the cold storage of food of animal origin. He developed a method for the rapid precooling and thawing of meat, the so-called one phase meat freezing.

As regards raw materials of plant origin, since 1949 he directed the selection of varieties suitable for quick-freezing, their analysis and their acclimatization in Hungary. After clarifying the relevant scientific and technical problems the experiments in technology were carried out often under his personal supervision. As a result, Hungary was one of the first countries to introduce quick-freezing of fruit and vegetables in freezing tunnels. It is partly due to this work that the Hungarian Refrigeration Industry was able to start an independent industrial activity in the field of quick-freezing of food.

The research work he has carried out on the laws on loss of mass in foods in the course of cooling, freezing and storage was equally valuable from the point-of-view of theory and practice. The results of these studies of physico-chemical character were utilized in the construction of the cold store in Szigetcsép, of the University of Horticulture and in the introduction of storage in controlled atmosphere at the same place. Another subject he paid much attention to was the development of the production technology of fruit juices and concentrates.

He was highly interested in the application of lyophilization in food preservation. Partly with an equipment of his own construction, partly with imported equipment he developed the production technology of about 80 food items. Of these lyophilized products coffee was introduced into industrial production.

As an acknowledgement of the results of his research work in 1958 he was awarded the title of Candidate of Chemical Sciences and in 1980 he received the degree of Doctor of Chemical Sciences.

The scientific work Elemér Almási has done was always closely connected to the daily tasks of food production and industrial activity and was aimed

at the development of technology. He was a permanent adviser to the Hungarian Refrigeration Enterprise. As an expert on the Board of Technical Development he contributed to many development programs. For several years he represented the Hungarian refrigeration industry in the Council of Mutual Economic Assistance. In the possession of the knowledge of several European languages he took part in many international conferences and study tours. He was granted various fellowships in the United Kingdom and in France to study modern methods of food preservation.

The results of his work were published in 140 papers. He was the author or co-author of two technical books and 8 university lecture notes. One of his books was published in the USSR. On the other hand, by translating 9 technical books from the Russian he laid the foundations of the Hungarian technical literature in several branches of the food industry.

Elemér Almási exerted a wide activity in Hungarian and foreign associations. He was vice-president of one of the committees of the International Institute of Refrigeration. He participated in the work of several complex committees of the Hungarian Academy of Sciences, such as Engineering and Chemistry, Bioengineering and Food Science. He was a member of the National Presidium and Board of the Hungarian Scientific Society for Food Industry.

His successful educational and research work was acknowledged by the many decorations conferred upon him. He was the owner of the commemorative medals founded in memory of Gábor Török, Elek 'Sigmond, Ferenc Entz and István Győry. In 1980 he was awarded the State Prize. On 4 April, 1985, in acknowledgement of his efforts pertinent to the development of the University of Horticulture he was awarded the Jubilee Honorary Diploma. As a man he alloyed his natural endowments with extreme industry, disciplined mode of living and purposeful application to his fields of interest. He was also a man of congenial disposition and serene, ready to help all who turned to him. In difficult situations he helped by giving wise advice without making the partner feel his superiority.

These characters made him popular with his colleagues and students. He was also a family man, devoted husband and father and a loving grandfather.

His memory is cherished with respect and he is set up as an example to the new generations of students.

K. Gasztonyi

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*Acta Alimentaria, Vol. 14 (4), pp. 303—308 (1985)*

# ANALYSIS OF CUBAN GRAPEFRUIT PEEL OIL

M. CORREA, R. TAPANES and J. PINO

National Center for Scientific Researches (CENIC), P.O.Box 6880, Havana, Cuba

(Received: 8 March 1983; revision received: 28 August 1984
accepted 4 October 1984)

The qualitative and quantitative analysis of the Cuban grapefruit peel oil was carried out by gas liquid chromatography, column chromatography and chemical separation techniques. As a result, 32 compounds were identified, including: 12 hydrocarbons, 8 carbonyl compounds and 8 alcohols. The physical and chemical properties of the essential oil are also given.

**Keywords:** Grapefruit, analysis of essential oils, gas liquid chromatography

Grapefruit peel oil has been widely studied in other areas (KESTERSON & HENDRICKSON, 1963; HUNTER & MOSHONAS, 1966; ZIEGLER, 1971) and these previous works have resulted in the identification of over 65 compounds (SHAW, 1977).

Because of the commercial importance of citrus peel oils in our country, this laboratory initiated investigations aimed towards extending knowledge of the composition of these essential oils (PEREZ et al., 1971, 1977, 1980; TAPANES & PEREZ, 1974; ARMENTEROS & PEREZ, 1978). The purpose of this study was to identify the volatile compounds of Cuban grapefruit peel oil.

## 1. Materials and methods

### *1.1. Samples*

The grapefruit coldpressed peel oil (2 kg) was obtained in a local factory and was representative of 1 ton of essential oil from the middle of the harvest season. The essential oil was extracted from red and white grapefruit varieties in unknown proportions with an industrial extractor "FMC-in line".

### *1.2. Analytical methods*

The physical and chemical characteristics of the essential oil were determined according to established standards (CUBAN STANDARD, 1967).

For the study of the essential oil composition the column-chromatographic technique with silica gel (70–230 mesh, activity 2–3; Merck, FRG) was used in a ratio essential oil — adsorbent 1 : 10. The essential oil (50 g) was eluted with hexane (2000 $cm^3$) to separate the hydrocarbons from the oxygenated compounds eluted with methanol (2000 $cm^3$). Elution with each solvent was continued until no more substances were being eluted, as shown by the



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refractive index of the eluent. Solvents were removed from each fraction in a vacuum evaporator (2.7 kPa). The hydrocarbon fraction was distilled with a fractionating column (40–60 °C, 400 Pa) in order to separate the monoterpenic and sesquiterpenic hydrocarbons.

Isolation of the organic groups present in the oil was made by chemical separation methods in order to obtain the $\alpha,\beta$-unsaturated aldehydes, aldehydes and alcohols by reaction with $Na_2SO_3$, $NaHSO_3$ and liquid–liquid extraction, respectively (HUNTER & MOSHONAS, 1965; TAPANES, 1975; ARMENTEROS & PEREZ, 1978; PINO, 1980).

The analyses by gas liquid chromatography were carried out using Packard-Becker 419 and Chrom 4 chromatographs. In the first one, different types of stainless steel columns (2.5 m × 2 mm) were used, packed with either 10% PEGA (British Drog House BDH, England,) or 15% DEGS (BDH) on 80–100 mesh Chromosorb W. In the second one, stainless steel columns (2.5 × 2 mm) were used with 10% PEG 20M (BDH) on 80–100 mesh Chromosorb W. The temperature was 80 °C initially and was raised to 200 °C at 5 °C $min^{-1}$, and kept isothermally at 200 °C. The flame ionization detectors operated at 220 °C and the argon carrier gas flow rate was 30 $cm^3$ $min^{-1}$.

The qualitative analysis was made by comparison of relative retention times on different columns and by the enrichment technique of chromatographic peaks, while the quantitative analysis was made by measuring the peak areas by graphical triangulation and calculating the relative areas. Means and standard deviations were results of triplicate measurements.

## 2. Results

The physical and chemical characteristics determined in the Cuban grapefruit peel oil are detailed in Table 1. These results allow us, on one side, to know better the composition of the essential oil that at present is produced,

Table 1

*Physical and chemical properties of the Cuban grapefruit peel oil*

| Property | Measured values | |
|---|---|---|
| | $\bar{x}$ | $\pm s$ |
| Relative density ($d_{25}^{25}$) | 0.8539 | 0.0005 |
| Refractive index ($n_D^{20}$) | 1.4770 | 0.0002 |
| Optical rotation ($\alpha_D^{20}$) | 92.7 | 0.3 |
| Evaporation residue (w/w %) | 6.88 | 0.02 |
| Aldehydes (w/w %, as decanal) | 1.27 | 0.01 |
| Esters (w/w %, as geranyl acetate) | 4.40 | 0.02 |

$\bar{x}$ = mean values
$\pm s$ = standard deviation

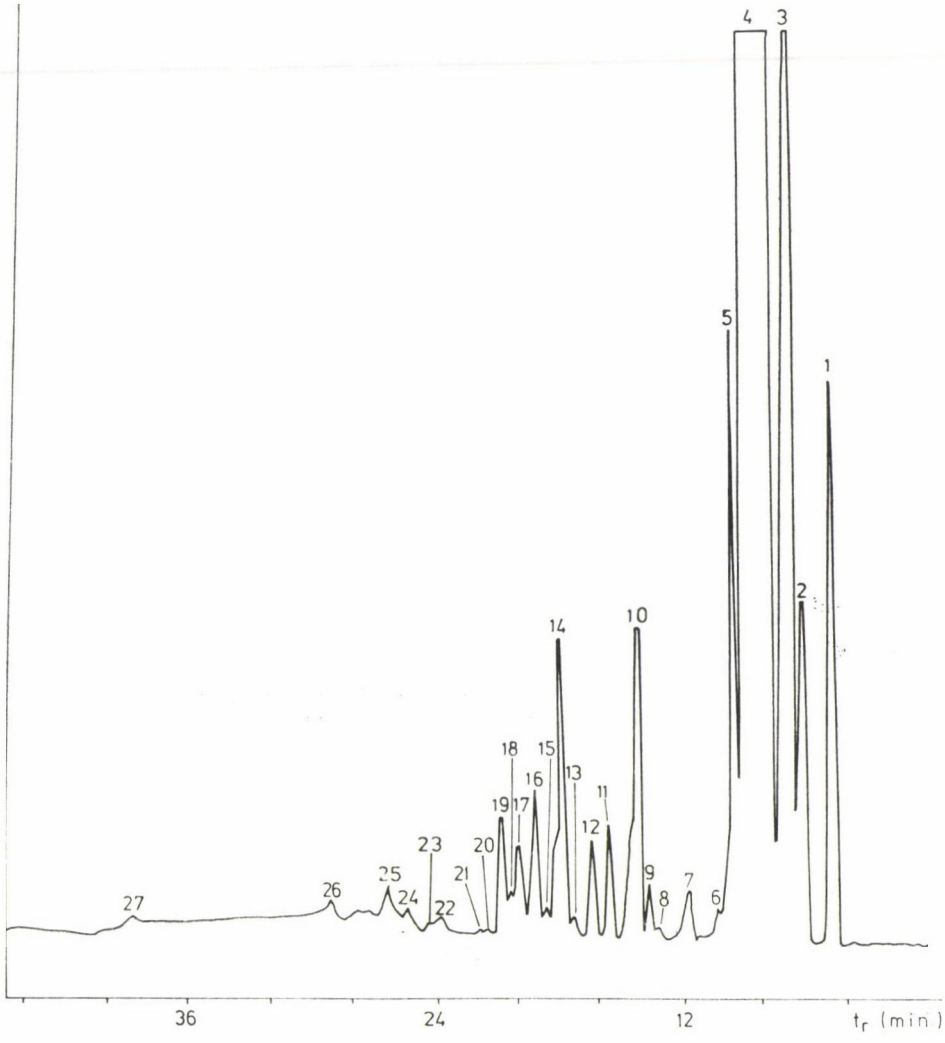


Fig. 1. Analysis by gas liquid chromatography of the Cuban grapefruit peel oil on PEG 20M column. Temperature program: 80–200 °C at 5 °C $min^{-1}$ and isothermically at 200 °C

and on the other hand, it is another way of evaluating the quality of the essential oil. The determined values agree with the reported values for the Florida grapefruit peel oil (KESTERSON et al., 1971).

A typical gas chromatogram of the essential oil is shown in Fig. 1. The great complexity in the composition is evident, although the higher quantity of the monoterpenic hydrocarbon limonene is also noticeable (chromatographic peak No. 4).

The separation by column-chromatographic technique made possible the simplification of the sample and also getting to know the actual ratio of hydrocarbons to oxygenated compounds, the result of which is 89.4% of hydrocarbons and 9.4% of oxygenated compounds (in the experiments, there was a loss of 1.2% of the essential oil).

The hydrocarbons were fractionated by distillation in two fractions: monoterpenic and sesquiterpenic hydrocarbons, which were analyzed by gas liquid chromatography. Results are summarized in Table 2.

The analysis of oxygenated compounds was carried out with the aid of chemical separation methods. In order to know the composition of the major carbonyl compounds, they were isolated quantitatively by reaction with $Na_2SO_3$ and $NaHSO_3$. The isolation of $\alpha, \beta$-unsaturated aldehydes showed the presence of neral and geranial, according to the gas-liquid-chromatographic analysis. Table 3 summarizes the results of the gas-liquid-chromatographic analysis of isolated aldehydes and ketones. The major carbonyl compounds of the essential oil are aldehydes and the major compounds are decanal and octanal.

The results of gas-liquid-chromatographic analysis of the isolated alcohols are shown in Table 4. In this fraction, the sesquiterpenic ketone nootkatone

Table 2

*Analysis by gas liquid chromatography of the terpenic hydrocarbons present in Cuban grapefruit peel oil*

| Peak No. | Compound | Relative area (%) | |
|---|---|---|---|
| | | $\bar{x}$ | $\pm s$ |
| | Monoterpenic hydrocarbons | | |
| 1 | α-pinene | 2.1 | 0.1 |
| 2 | sabinene | 1.5 | 0.1 |
| 3 | mircene | 6.7 | 0.2 |
| 4 | limonene + γ-terpinene | 89.6 | 0.4 |
| | Sesquiterpenic hydrocarbons | | |
| 1 | α-copaene | 1.4 | 0.1 |
| 2 | — | 1.0 | 0.1 |
| 3 | α-ilangene | 20.0 | 0.2 |
| 4 | β-copaene | 12.7 | 0.2 |
| 5 | β-elemene | t | |
| 6 | cariophylene | 29.0 | 0.3 |
| 7 | — | 6.3 | 0.2 |
| 8 | humulene | 7.7 | 0.2 |
| 9 | — | 5.9 | 0.2 |
| 10 | Δ-cadinene | 13.0 | 0.3 |
| 11 | — | t | |
| 12 | — | 1.0 | 0.1 |
| 13 | — | 1.4 | 0.1 |
| 14 | — | t | 0.1 |

$\bar{x}$ = mean values
$\pm s$ = standard deviation
t = in traces (less than 1%)
conditions of analysis: 10% PEGA;
temperature program: 80–200 °C at 5 °C $min^{-1}$, and isothermally at 200 °C;

Table 3

*Analysis by gas liquid chromatography of saturated aldehydes present in Cuban grapefruit peel oil*

| Peak No. | Compound | Relative area (%) | |
|---|---|---|---|
| | | $\bar{x}$ | $\pm s$ |
| 1 | octanal | 14.8 | 0.2 |
| 2 | nonanal | 4.7 | 0.1 |
| 3 | — | 1.2 | 0.1 |
| 4 | decanal | 71.8 | 0.4 |
| 5 | undecanal | 1.9 | 0.1 |
| 6 | dodecanal | 5.5 | 0.2 |

$\bar{x}$ = mean values
$\pm s$ = standard deviation
conditions of analysis: 10% PEGA;
temperature program: 80–200 °C at 5 °C $min^{-1}$, and isothermally at 200 °C

Table 4

*Analysis by gas liquid chromatography of the alcohols present in Cuban grapefruit peel oil*

| Peak No. | Compound | Relative area (%) | |
|---|---|---|---|
| | | $\bar{x}$ | $\pm s$ |
| 1 + 2 | linalool + octanol | 50.2 | 0.4 |
| 3 | — | 1.2 | 0.1 |
| 4 | nonanol | 1.7 | 0.1 |
| 5 | decanol + α-terpineol | 17.4 | 0.3 |
| 6 | — | 5.5 | 0.2 |
| 7 | citronelol | 4.4 | 0.1 |
| 8 | nerol | 4.0 | 0.1 |
| 9 | geraniol | 3.1 | 0.1 |
| 10 | — | 1.8 | 0.1 |
| 11 | — | 1.3 | 0.1 |
| 12 | — | t | |
| 13 | — | t | |
| 14 | — | 3.3 | 0.1 |
| 15 | — | t | |
| 16 | nootkatone | 5.9 | 0.2 |

$\bar{x}$ = mean values
$\pm s$ = standard deviation
t = in traces (less than 1%)
conditions of analysis: 10% PEGA;
temperature program: 80–200 °C at 5 °C $min^{-1}$ and isothermally at 200 °C;

was isolated in agreement with the results reported in the original method (HUNTER & MOSHONAS, 1965). The major alcohols of the essential oil are linalool and α-terpineol.

## 3. Conclusions

Physical and chemical properties of the Cuban grapefruit peel oil agree with the values reported for the Florida essential oil. The Cuban essential oil shows 89.4% of terpenic hydrocarbons and 9.4% of oxygenated compounds.

In the present study, 40 compounds were isolated, 26 of which have been identified with the aid of gas liquid chromatography. The elucidation of the other unknown compounds and the quantitative analysis of the total composition are in progress.

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# ATTEMPTS TO DETERMINE PROTEIN, FAT AND MOISTURE IN "ANIMAL PROTEIN MEAL" BY THE NIR TECHNIQUE

K. J. KAFFKA[a] and A. P. MARTIN[b]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] Enterprise for Production of Feeds from Animal By-products, H-1097 Budapest, Illatos út 23. Hungary

(Received: 28 October 1983; revision received: 6 August 1984; accepted: 10 September 1984)

Preliminary studies were performed on animal protein meal samples with different compositional values in order to establish how composition parameters, especially protein, fat and moisture content, can be determined with reflectance measurements in the near infrared wavelength region from 1100 to 2500 nm, and to estimate the accuracy in predicting the chemical constituents of animal protein meal using the instrumental method mentioned.

A Neotec 6450 type research composition analyzer was used to study the animal protein meal samples labelled with the composition parameters determined by traditional (wet-chemistry) standard methods.

The negative logarithm of the near infrared reflectance factor ($R$) spectra were recorded for the 27 animal protein meal samples. The log ($1/R$) spectra were then transformed to the second derivatives versus wavelength for correlation with compositional data. Iterative linear regression technique was used to determine the optimum wavelengths and other parameters for predicting each of the chemical constituents.

The derivative NIR technique applied to animal protein meal provides single term prediction equations — using the ratio of second derivatives of log ($1/R$) measured at two characteristic wavelengths — giving correlation coefficients of 0.993 for protein, 0.988 for fat, and 0.990 for moisture. In a measurement time of less than 30 seconds it was possible to determine protein content within a standard error of calibration of $\pm 1.225$ mass %, fat content within $\pm 0.938$ mass %, and moisture content within $\pm 0.438$ mass %.

The above described studies led to the conclusion, that in the case of animal protein meal, the composition parameters of major importance can be predicted with a quick, accurate and non-destructive instrumental method using the derivative NIR technique.

**Keywords:** Animal protein meal analysis, NIR technique, protein, fat and moisture determination

With the development of animal breeding it is increasingly important to use fodder containing animal protein. These products of high protein content can bring essential amino acid content of fodder mixtures to the level required by the species and breeding purpose.

In order to fulfil higher demands it is important to provide production and control with as many accurate and promptly available compositional data as possible. We studied the possibilities of determining the chemical composition of animal protein meal using optical properties measured by quick, non-destructive methods.

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Near infrared reflection (NIR) spectroscopy is a rapid, non-destructive efficient method already used for determining constituents of several agricultural and food industrial products. This spectroscopic technique was elaborated in order to substitute the wide-spread wet-chemistry analytical methods which are very time and labour consuming. Instead of the Kjeldahl method for determining protein content, solvent extraction method for determining fat or oil content, respectively, and for substituting oven-drying method for moisture content determination NIR technique is gaining even wider application. NIR technique is based on the interaction of the sample with infrared radiation; namely a beam transmitted or reflected by a sample contains information on the composition of the sample. A large number of food components have absorption peaks in the near infrared spectral region of 1000–2500 nm; therefore, this region is particularly useful for determining the composition of food products.

BEN-GERA and NORRIS (1968) already dealt with determining fat and moisture content in meat products using NIR technique. The linear equations established between the composition and the transmittance data, measured at two wavelengths with the special spectrophotometer built by the authors, described the moisture content in the region of 45 to 75 mass % with an error of 1.4 mass %, and the fat content in the region of 5 to 35 mass % with an error of 2.1 mass %. The main sources of error were found to be deviations in sample thickness and the low signal to noise ratio due to high absorption. By reflection measurements both errors can be reduced.

Later ROSENTHAL (1973) reported a single-purpose instrument using the near infrared reflection technique for the rapid, non-destructive determination of fat content in minced meat products. MASSIE (1976) developed an instrument using Ga-Ar emitter for the same purpose.

In the nineteen seventies the serial production of certain single-purpose instruments based on NIR technique began. HAUSER and WEBER (1978, 1980) reported experience in the field of determining water, fat, moisture, protein and ash using commercially available single-purpose instruments.

KRUGGEL and co-workers (1981) as well as ARNETH (1982) used Infra-Alyser-400 for determining composition of meat products.

MARTENS and co-workers (1982) revealed relationship between composition parameters of meat products and their reflection values at different discrete wavelengths using novel mathematical methods.

LANZA (1982) and NÁDAI (1983) used the Neotec 6450 RCA for studying raw beef and pork samples; the former also studied correlation between calory content and composition whereas the latter primarily studied the influence of disturbing factors such as sample thickness, positioning, temperature and particle size on accuracy.

As seen, NIR technique has been used for meat and meat products by a number of researchers.

On the basis of the above results, we decided to study the applicability of NIR technique for determining the composition of animal protein meal. So far, such measurements have not been reported yet in the literature.

The present study aims at finding the possibility for applying the NIR technique for the rapid determination of protein, fat and moisture content in animal protein meal and, at the same time, to predict accuracy for the different components, to choose the most suitable form of the regression equations, to determine the parameters (characteristic wavelengths, coefficients, and constants) by studies on the transformed reflectance spectra.

The results of these studies will hopefully enable us to develop a single-purpose instrument or to extend the use of another one to be capable to perform such measurements.

## 1. Materials and methods

The main raw materials of animal protein meal are provided by slaughterhouses, poultry processing factories. At the moment there is no possibility to separate raw materials into "meat-meal", "blood-meal", "bone-meal", etc., thus they are mixed and processed together. Consequently our experimental samples were also of mixed composition, however, we have taken care to represent the peculiarities of different production regions (Debrecen, Hódmező-vásárhely, Szikszó, Solt, Szolnok, Tököl, Sárvár) and the different processing technologies — moreover we even added blood meal and keratine to our calibration samples to make the picture complete.

The products are priced according to their raw protein content, while microbiological conditions are determined by moisture content, whereas fat content influences shelf life. During our investigations we restricted ourselves to establish protein, fat and moisture content with the help of NIR technique.

Determinations for moisture, fat and protein content are described in the HUNGARIAN STANDARDS (1977, 1978, 1981).

The relevant HUNGARIAN STANDARD (1980) generally deals with feedstuffs of animal origin.

The optical properties of animal protein meal samples were measured with a Neotec 6450 research composition analyzer (RCA). In his paper LANDA (1979) gave a detailed report of this instrument. The log $(1/R)$ spectra in the 1100–2500 nm wavelength range were measured in 2 nm steps and were stored for further processing on a floppy-disc.

$R$ is the conical-conical reflectance factor which is the ratio of the flux actually reflected by the sample to the one which is reflected into the same

reflected-beam geometry by an ideal (lossless), perfectly diffuse (Lambertian) standard surface irradiated in exactly the same way as the sample.

Preliminary studies indicated, as NORRIS and KAFFKA (1982) reported, that the performance could be improved by using the second derivative of the log $(1/R)$ spectral curve. Therefore we transformed the data to the second derivative of the log $(1/R)$ spectra. The relationships between transformed spectral data and composition parameters were studied in the following form of equations

$$Q_i = K_{1i} + K_{2i} \frac{V''_{\lambda 1i}}{V''_{\lambda 2i}}$$

where

$Q_i$ stands for the composition parameters;
$K_{1i}$ and $K_{2i}$ are constants and coefficients, resp.;
$V''_{\lambda 1i}$ and $V''_{\lambda 2i}$ are the values of the second derivatives of the log $(1/R)$ spectra at $\lambda_{1i}$ and $\lambda_{2i}$ characteristic wavelengths.

Characteristic wavelengths, constants and coefficients of these single term regression equations, as well as standard errors of calibration and correlation coefficients, are summarized in Table 2.

The definition of the standard error of calibration used is:

$$\sqrt{\frac{\sum_{i=1}^{n} (Q_{si} - Q_{ci})^2}{n - 1 - p}}$$

where

$n$ is the number of samples;
$Q_s$ is the respective quality parameter (dependent variable) determined by traditional standard methods;
$Q_c$ is the same quality parameter computed from the regression equation;
$p$ is the number of independent variables.

## 2. Results

The compositional data — namely protein, fat and moisture content — of the 27 animal protein meal samples are summarized in Table 1.

We selected the log $(1/R)$ spectra of two samples; one with relatively high fat content and one (a blood meal sample) with high protein content, which are shown in Figs. 1 and 2. The second derivative curves of the corresponding log $(1/R)$ spectra are also plotted in the same figures.

The Figures illustrate that the second derivatives of the log $(1/R)$ spectra show much greater difference than log $(1/R)$ between samples with different composition, sharpening the details in the curves.

Table 1

*Compositional data of the 27 animal protein meal samples*

| Sample No. | Protein content (mass %) | Fat content (mass %) | Moisture content (mass %) |
|---|---|---|---|
| 1 | 68.2 | 5.8 | 2.1 |
| 2 | 65.6 | 5.9 | 5.6 |
| 3 | 65.8 | 5.9 | 5.6 |
| 4 | 64.4 | 5.6 | 7.6 |
| 5 | 62.9 | 5.6 | 11.0 |
| 6 | 59.3 | 19.9 | 2.4 |
| 7 | 56.5 | 19.4 | 6.0 |
| 8 | 57.2 | 18.9 | 6.2 |
| 9 | 56.6 | 16.8 | 7.4 |
| 10 | 54.8 | 20.1 | 10.6 |
| 11 | 63.8 | 15.3 | 2.7 |
| 12 | 60.8 | 14.9 | 6.4 |
| 13 | 60.3 | 15.1 | 6.5 |
| 14 | 59.5 | 15.1 | 8.2 |
| 15 | 57.8 | 14.0 | 11.8 |
| 16 | 57.1 | 17.1 | 2.3 |
| 17 | 55.3 | 17.1 | 6.2 |
| 18 | 54.8 | 16.2 | 7.3 |
| 19 | 54.6 | 19.2 | 8.2 |
| 20 | 53.2 | 15.5 | 10.0 |
| 21 | 59.2 | 16.5 | 2.4 |
| 22 | 56.8 | 16.1 | 5.5 |
| 23 | 56.7 | 16.3 | 5.8 |
| 24 | 56.0 | 15.8 | 7.6 |
| 25 | 54.3 | 17.5 | 11.7 |
| 26 | 90.7 | 0.20 | 9.6 |
| 27 | 97.8 | 0.14 | 2.0 |

Table 2

*Summary of linear regression analyses relating data from chemical analyses and values of the second derivative of log (1/R) curves at two characteristic wavelengths for 27 animal protein meal samples, searching 1100 to 2500 nm*

Equation form: $Q_i = K_{1i} + K_{2i}\left(\frac{V''_{\lambda 1 i}}{V''_{\lambda 2 i}}\right)$

| | For protein content | For fat content | For moisture content |
|---|---|---|---|
| Characteristic wavelength $\lambda_1$ (nm) | 1770 | 1204 | 1826 |
| Characteristic wavelength $\lambda_2$ (nm) | 2340 | 1330 | 2184 |
| Constant $K_1$ | 40.079 | 2.133 | 8.280 |
| Coefficient $K_2$ | −34.623 | −6.751 | −22.186 |
| Segment (wvl range for averaging) (nm) | 4 | 4 | 4 |
| Gap for numerator (nm) | 12 | 12 | 12 |
| Gap for denominator (nm) | 12 | 12 | 12 |
| Standard error of calibration (mass %) | 1.225 | 0.938 | 0.438 |
| Correlation coefficient | 0.993 | 0.988 | 0.990 |

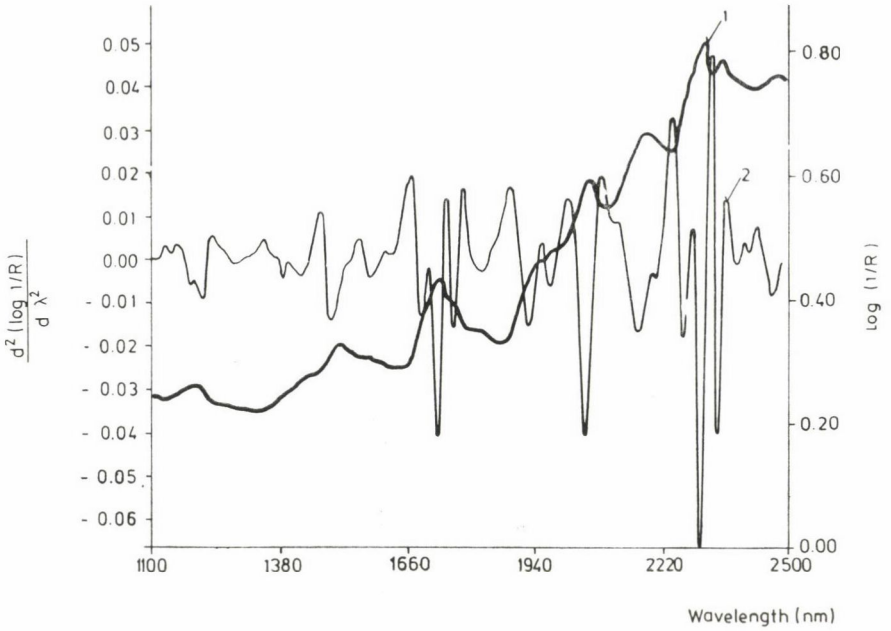


Fig. 1. The log $(1/R)$ spectrum and the second derivative of animal protein meal sample No. 6 containing high percentage of fat. The measuring geometry: 0°/45°. Diameter of the illumination beam: 25 mm. Spectral bandpass: 10 nm. Spectra were taken in 2 nm steps. ——: log $(1/R)$ spectrum of sample No. 6, – – –: second derivative of the log $(1/R)$ spectrum

Table 3

*Other wavelength-pair possibilities to determine protein, fat and moisture content in animal protein-meal*

Equation form: $Q_i = K_{1i} + K_{2i}\left(\frac{r^*_{\lambda 1 i}}{r^*_{\lambda 2 i}}\right)$

| | $\lambda_1$ (nm) | $\lambda_1$ (nm) | SEC | $R$ |
|---|---|---|---|---|
| For protein | 1746 | 2216 | 1.258 | 0.993 |
| | 2178 | 2368 | 1.554 | 0.989 |
| | 2044 | 2330 | 1.746 | 0.986 |
| For fat | 1756 | 2186 | 1.173 | 0.981 |
| | 2318 | 2130 | 1.199 | 0.980 |
| | 2342 | 2188 | 1.319 | 0.976 |
| For moisture | 1368 | 1642 | 0.532 | 0.985 |
| | 1454 | 1338 | 0.539 | 0.984 |
| | 1860 | 1658 | 0.563 | 0.982 |

SEC: standard error of calibration
$R$: near infrared reflectance factor

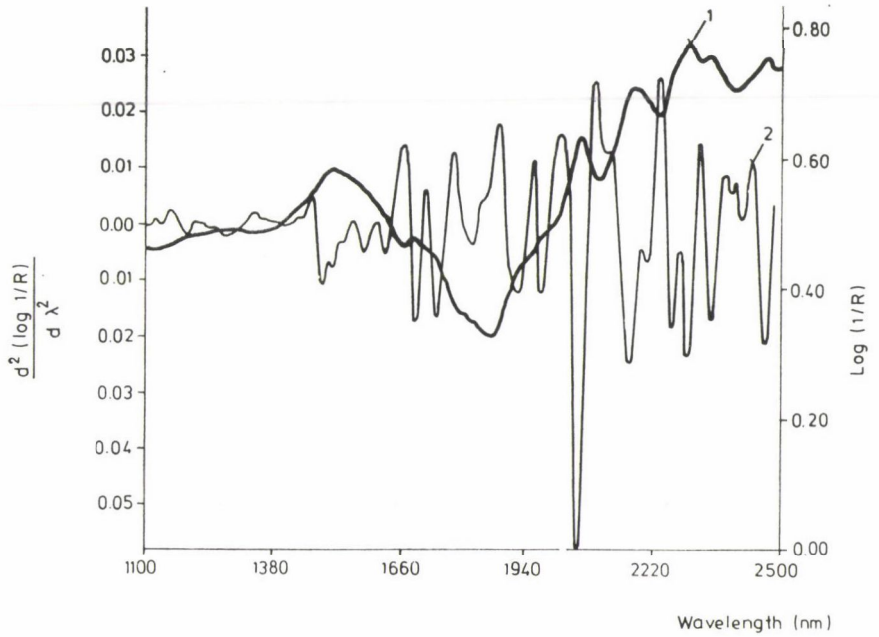


Fig. 2. The log ($1/R$) spectrum and the second derivative of animal protein meal sample No. 27 (blood meal) containing high percentage of protein. The measuring geometry: 0°/45°. Diameter of the illumination beam: 25 mm. Spectral bandpass: 10 nm. Spectra were taken in 2 nm steps. ▬▬: log ($1/R$) spectrum of sample No. 27, ——— : second derivative of the log ($1/R$) spectrum

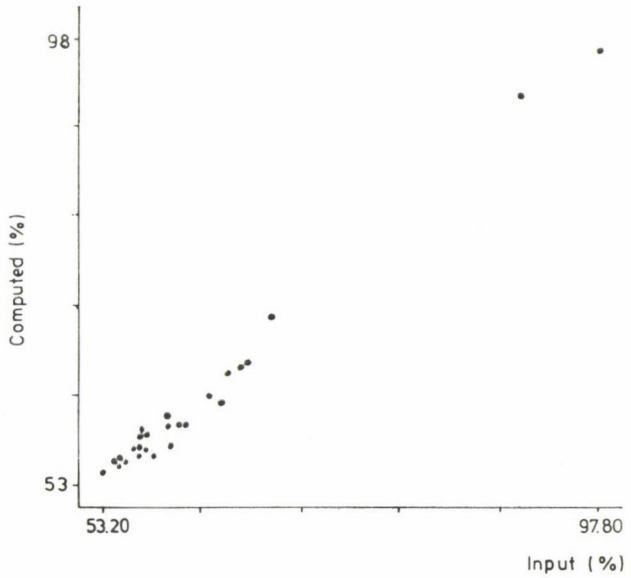


Fig. 3. Relationship between protein content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log ($1/R$) curves at two characteristic wavelengths

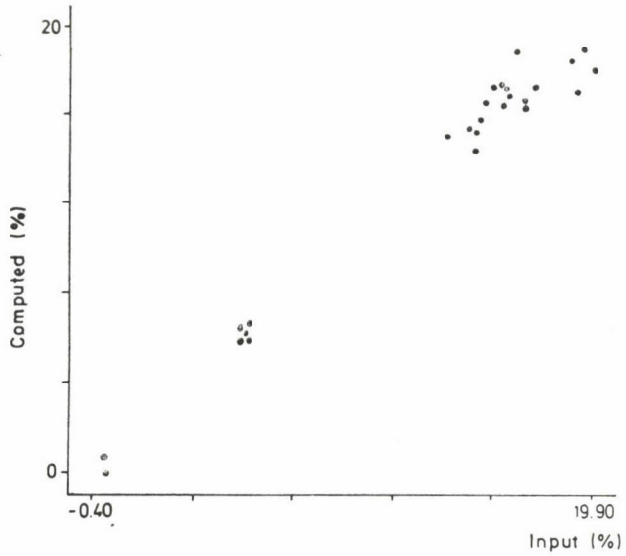


Fig. 4. Relationship between fat content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log $(1/R)$ curves at two characteristic wavelengths

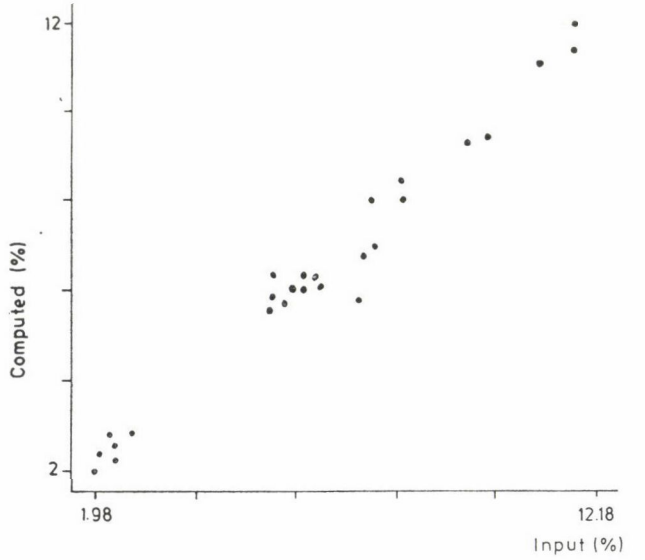


Fig. 5. Relationship between moisture content determined by chemical analysis and predicted value from linear regression of the ratio of the second derivatives of the log $(1/R)$ curves at two characteristic wavelengths

The relationship between optical properties (the second derivative values of the log $(1/R)$ spectra) and compositional data (protein, fat and moisture content) of the 27 animal protein meal samples were determined by the use of an iterative procedure with the linear regression analyses.

Results are summarized in Table 2. In this Table, the gaps for numerator and denominator, wavelengths, as well as the segments, are listed. The gaps are the distances among the three wavelengths used for producing the second derivative, while the segment is the wavelength region in which the measured spectral data are averaged.

The results for protein, fat and moisture determination, using the derivative NIR technique with the single-term equations shown in Table 2 are plotted in Figs. 3–5. Our studies showed that acceptable results can be achieved at other wavelength-pairs, too; some of them together with their respective standard errors are listed in Table 3.

## 3. Conclusion

As can be seen in Table 1 the protein content in our animal protein meal samples lies in the range 53.5–97.8 mass %. The range of fat content is 0.14–19.9 mass %. Moisture contents fall in the range of 2.0–11.8 mass %.

The particle size distribution of the samples was not tested, but it was easy to establish that the range of particle sizes was also very broad as a consequence of the different producing technologies used.

Although the ranges of mass % for all the investigated components as well as of particle size were extremely broad, satisfactory results were obtained for protein, fat and moisture content of animal protein meal samples using the NIR technique.

Animal protein meals produced in Hungary are mixtures derived from raw materials the composition of which can not be influenced. The amino acid composition of proteins, the fatty acid composition of fats, and the bounding energy levels of water in the raw materials are different. Thus it seems evident that animal protein meal is explicitly of many components and is expected that the equation for determining composition using the measurement of optical properties must be multiterm. The number of carefully analyzed calibration samples at hand did not open a possibility for generating equation with several terms without the danger of overfitting and loosing the value of statistical parameters.

In our experiments we actually used a single term equation where the value of the second derivative of the log $(1/R)$ spectra at the first characteristic wavelength was divided by the value of the second derivative of the log $(1/R)$ spectra at the second characteristic wavelength.

The calibration results have not been tested against unknown samples, but the very high correlation coefficients indicate, that it should be possible to predict protein, fat and moisture content with high accuracy using near infrared reflectance techniques.

These results were obtained by exploring only one of the many possible data treatments and using only a single-term equation and this single-term equation without optimizing the parameters (segment, gap in numerator, gap in denominator) in it.

The increase of the number of samples included, in determining the equation, is needed for increasing the number of terms — and this is our task for the future in order to increase accuracy.

*

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# CHARACTERIZATION OF THE COLOUR OF RED WINES BY TRICHROMATIC VALUES

A. KAMPIS and Á. ÁSVÁNY

Central Food Research Institute, H-1022, Budapest, Herman Ottó út 15. Hungary
National Institute for Wine Qualification, H-1027, Budapest, Bem tér 2. Hungary

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Correlations between the trichromatic values ($x$, $y$, $Y$) of red wine, determined by the tristimulus system suitable for objective colour measurement, and the traditionally applied characteristic colour parameter (absorbance as measured at 520 nm), are discussed.

In the course of the study the changes of the tristimulus values of red wines (Kékfrankos, 37 samples; Cabernet franc, 20 samples), obtained by heating technique from grapes grown at different locations in the years 1977, 1978 and 1979 were investigated during one year storage.

An inverse proportion was found between the $Y$ value expressing the lightness of colour as seen by the naked eye, and the absorbance of red wine at 520 nm according to our results evaluated by a mathematical statistical method.

Based on the correlation between $x$ and $y$ values of red wines from different production area and of different variety the locations of the sample points (of chromaticity coordinates $x$ and $y$) on the chromaticity diagram were determined.

The correlation between colour characteristics $Y$ and $x$ of red wines different locality and variety can be described by a hyperbola as established by the mathematical statistical evaluation of results.

By analysis of the changes of the tristimulus values of red wines during one year storage the tendencies of the shift in the chromaticity diagram of the sample points defined by the chromaticity coordinates $x$ and $y$ were determined. The colour points of the darkest musts located in the angle of the colour triangle moved parallel to the spectrum locus, while the hue shifted from purple towards orange-red. When the colour points reached the region limited by radial lines corresponding to wavelengths of 630 and 640 nm, remaining within the zone they shifted towards the white point.

**Keywords:** Colour determination, colour of red wine, colour measurement

The most striking characteristic of red wines is their colour therefore in the course of the evaluation of different red wine vinification techniques the objective, instrumental colour measurement is very important from the point of view of quality improvement and control.

The truly objective method of colour measurement is the determination of the tristimulus values as laid down in the C.I.E. Standard, 1931 (BERNOLÁK, 1971). Few data are available in the related literature on the use of the tristimulus system for the determination of wine colour. This is because the correlations between the trichromatic values ($x$, $y$, $Y$), or between them and other, optical and chemical colour characteristics are not clarified yet.

ROBINSON and co-workers (1966a) carried out a comprehensive study on the applicability of the tristimulus method for the colour measurement of



*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

wine. Changes of the colour of red wine and changes of the tristimulus values upon dilution and sulphiting were investigated. They characterized the stability of certain antocyanin compounds and their changes during storage by the tristimulus values (ROBINSON et al., 1966b). They found that during storage one of the tristimulus values, hue shows a definite shift from the violet-red region toward orange-red.

BALLINGER and co-workers (1974) investigated 39 clones of *Vitis rotundifolia* and by evaluating the results with computer they found a multivariable linear relationship between the natural logarithm of the total anthocyanin content of the grapes (and the concentrations of individual anthocyanins identified) on the one hand and the tristimulus values of the wines fermented from the grapes investigated on the other. LITTLE (1977) using the tristimulus method developed a new technique for the determination of pH dependent (monomer) and pH independent (polymer) anthocyanins in red wines. In the pertinent Hungarian literature only a single paper was published so far on the characterization of red wine colour by tristimulus values (MOLNÁR & LUKÁCS, 1978).

In the Research Institute for Viticulture and Oenology, Hungary the tristimulus method has been in use for the determination of red wine colour since 1976. By processing the large amount of measured data a connection was sought between the trichromatic values ($x$, $y$, $Y$) of red wines of different origin and different variety as well as between the $Y$ value and the traditionally applied colour parameter, absorbance at 520 nm in red wine calculated for optical pathway length of 1 cm ($A_{520}$ value). By establishing the correlation between the trichromatic values and the generally applied wine colour characteristic the authors aimed at being able to evaluate the tristimulus values measured in relation to the colour characteristic used widely in wine analysis.

During storage of red wine the tristimulus values change. We followed up these changes in red wines obtained by heating technique during one year storage in three consecutive years to observe the trends in the shift of tristimulus values with colour changes as seen by the naked eye.

## 1. Materials and methods

### *1.1. Materials*

*1.1.1 Red wines of 1976 from different locations of cultivation and of different varieties.* To establish correlations between the tristimulus values and between $Y$ values measured by tristimulus method and the absorbance measured at 520 nm we used data of red wines of different production area and of different varieties, prepared in 1976, measured after fermentation and after one year storage.

Varieties used and their place of cultivation:

— Kadarka from Kiskőrös,
— Kármin from Kiskőrös,
— Cabernet franc from Villány,
— Cabernet franc from Eger,
— Kékfrankos from Eger.

*1.1.2. Kékfrankos and Cabernet wines obtained by heating technique in the years 1977, 1978 and 1979.* The wines were prepared by heating technique from grapes cultivated at the Research Station in Eger of the Research Institute for Viticulture and Oenology. Varying the parameters of the heat treatment the mash was heated by using a Rosenblad (Alfa-Laval; 5 t per h capacity) heat exchanger to 50, 60 and 70 °C for holding times of 30, 60 and 120 minutes. Wines fermented on skins from grapes of the same variety were used as control. Batches of 20 kg of the heat treated mash were pressed three times in basket presses, then sulphited with $K_2S_2O_5$, equivalent to 50 mg $dm^{-3}$ $SO_2$ (Reanal, Budapest). To start fermentation selected yeast culture of Eger-1 was used. When fermentation finished the wine was drawn off and the free sulphurous acid content was made up to 50 mg $dm^{-3}$ with $K_2S_2O_5$. The wines were kept without any further treatment in demijohns for 1 year. The tristimulus values were determined in the musts, in the new wines after fermentation and in January and August of the next year.

## *1.2. Methods*

*1.2.1. Determination of tristimulus values.* In order to reach optical purity, red wine samples were centrifuged (Janetzky T-24, LGW, Leipzig) for 10 min at 250 r.p.m. The tristimulus values were determined by Momcolor-D (MOM, Hungarian Optical Works, Budapest) tristimulus colorimeter. The $X_2$, $X_1$, $Y$ and $Z$ values were determined on a pathway of 0.5 cm. The standard "C" light source was used. For calibration a white enamel etalon certified by the National Bureau of Measuring (Budapest) was used. The trichromatic values $x$, $y$, $Y$ were calculated from the average of three parallel measurements according to the standardized convention of C.I.E., 1931 (BERNOLÁK, 1971).

*1.2.2. Determination of the $A_{520}$ values.* In order to ensure optical purity the red wine samples were centrifuged for 10 min at 250 r.p.m. Absorption at 520 nm, calculated for optical pathway of 1 cm, was measured with Acta CIII (Beckman, California) spectrophotometer.

*1.2.3. Correlations between the various colour characteristics.* Correlation between the $Y$ value, corresponding to lightness as perceived by the naked eye, and the parameter generally used in wine analysis to characterize the colour: $A_{520}$ value was studied by analysing wine made in 1976 from grapes of different places of cultivation and of different varieties. The tristimulus

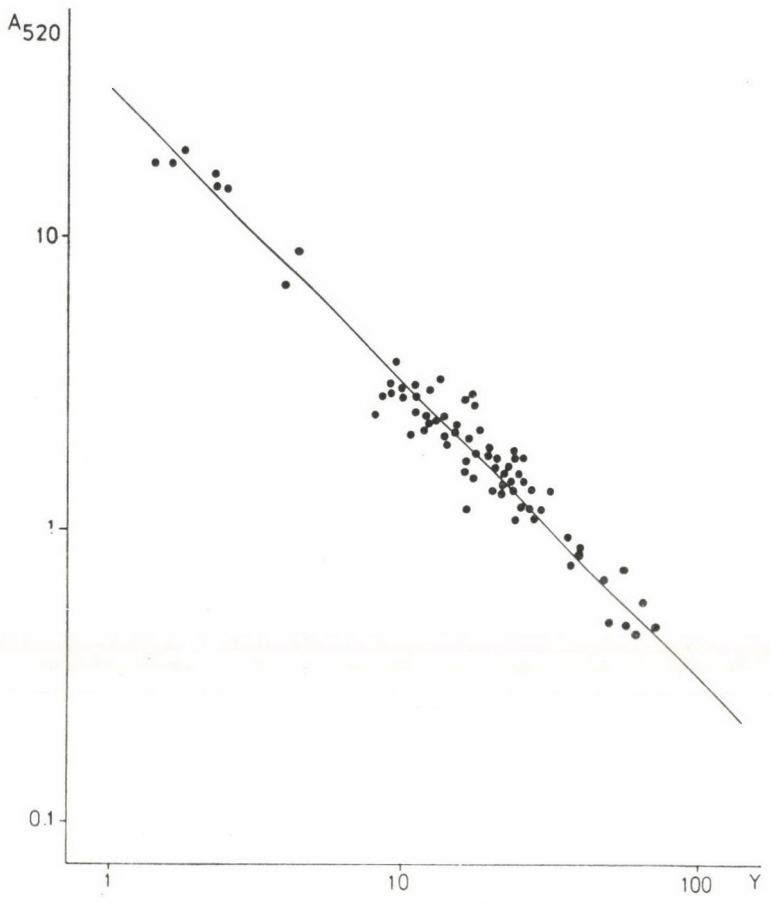


Fig. 1. Correlation between the logarithms of trichromatic value ($Y$) and absorbance at 520 nm ($A_{520}$) values of red wines of different variety grapes, harvested in 1976. $\log A_{520} = 1.501 - 1.02 \log Y$; $r = 0.96$; $n = 74$

values and the $A_{520}$ values were determined in wines directly after fermentation and after one year storage.

To analyze the connections between the tristimulus values of red wines the tristimulus values obtained in wines mentioned above data of three vintages of 1977, 1978, and 1979, measured and averaged after fermentation and after one year storage of Kékfrankos and Cabernet franc wines made by heating technique were plotted in the chromaticity diagram of coordinates $x$ and $y$. The $x, y$ coordinates of the clear, monochromatic colours of the spectrum form the horseshoe shaped spectrum locus in the chromaticity diagram. All existing colours are located within the spectrum locus. The white point corresponding to uniform illumination is in the middle of the curve (BERNOLÁK, 1971). The standard realms corresponding to colour names (PRETSCH, 1962),

the line of the full colours and the radial lines connecting the points of equal hue but different saturation are shown in the chromaticity diagram.

To follow up the changes during fermentation and storage the tristimulus values were determined in samples of Kékfrankos and Cabernet franc wines, made by heating technique in 1977, 1978 and 1979, samples from the must, from the new wine, from the wine once drawn and after one year storage.

## 2. Results

### *2.1. Correlation between the* Y *and* $A_{520}$ *values in red wines*

The $Y$ value is characteristic of light transmission, while the $A_{520}$ value shows the absorption of red light of 520 nm wavelength, thus, a negative correlation is expected between the two values. The results obtained in this study show a linear correlation between the logarithms of the two parameters (Fig. 1). The slope of the line is $-1.02$, thus, the $Y$ and $A_{520}$ values are in-

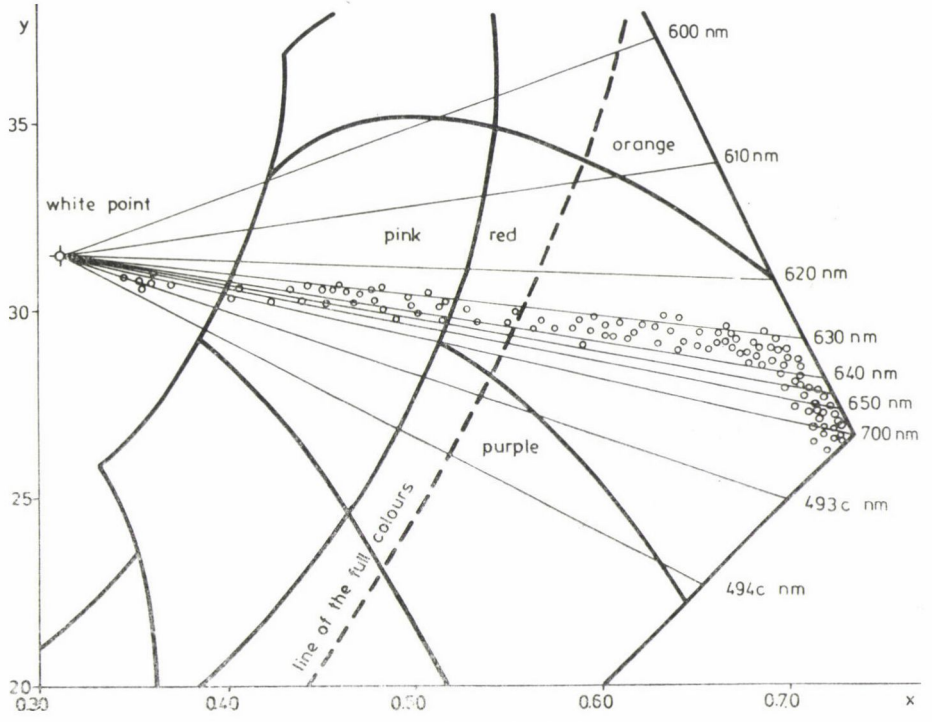


Fig. 2. Correlation between the $x$ and $y$ values of red wines, calculated by the tristimulus method, displayed in the chromaticity diagram ($n = 113$). Equation of the lines from the white point to the spectrum line: $y = 0.33 - 0.05\,x$; $r = 0.92$; $n = 82$

versely proportional to a close approximation. Equation of the hyperbola describing the inverse proportionality between the initial variables is:

$$A_{520} = \frac{31.7}{Y}.$$

*2.2. Correlation between the* x, y *values determined by the tristimulus method*

The tristimulus values were plotted in Fig. 2. Analysis of the results has shown the colour points (defined by coordinates $x$ and $y$) of the samples studied to be in the region between the lines corresponding to spectral colours of wavelengths of 630 and 640 nm. The points belonging to "rosé" wines start next to the white point (beginning with 8% saturation). With increasing saturation the colour points pass through the "pink" colour region, then intersect the line of the full colours, reach the spectrum locus i.e. the boundary of the colour triangle. Here the curve described by the colour points of the samples turns downwards and fills the corner of the triangle corresponding to

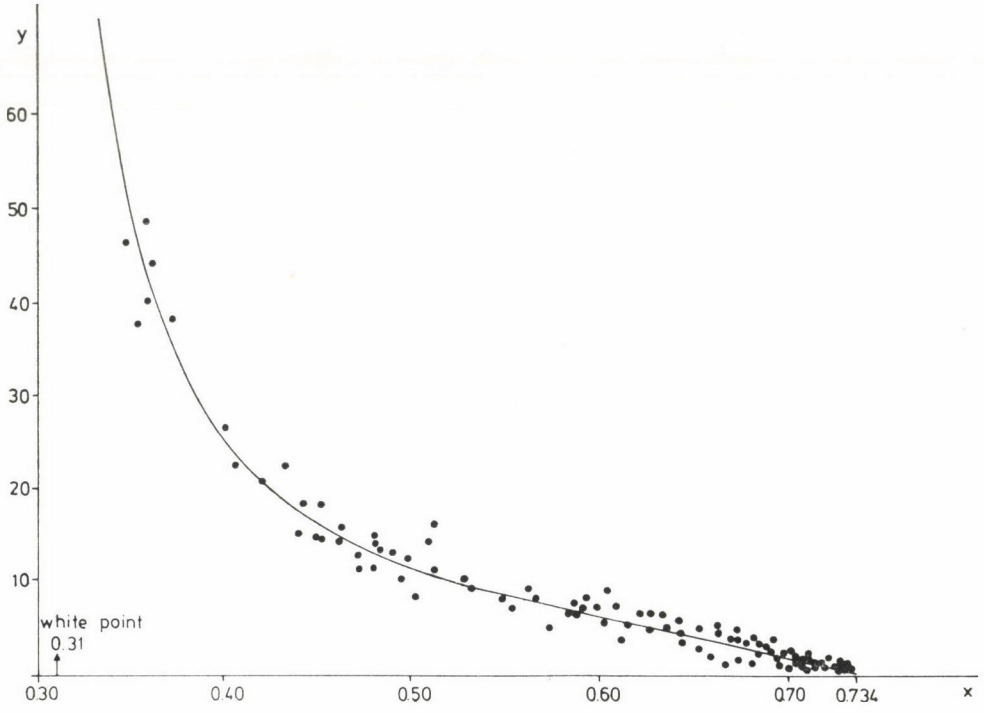


Fig. 3. Correlation between the colour characteristics $Y$ and $x$ as calculated by the tristimulus method. Equation of the hyperbolic correlation: $Y = 10.2 \frac{0.74 - x}{x - 0.26}$; $r = 0.93$; $n = 74$

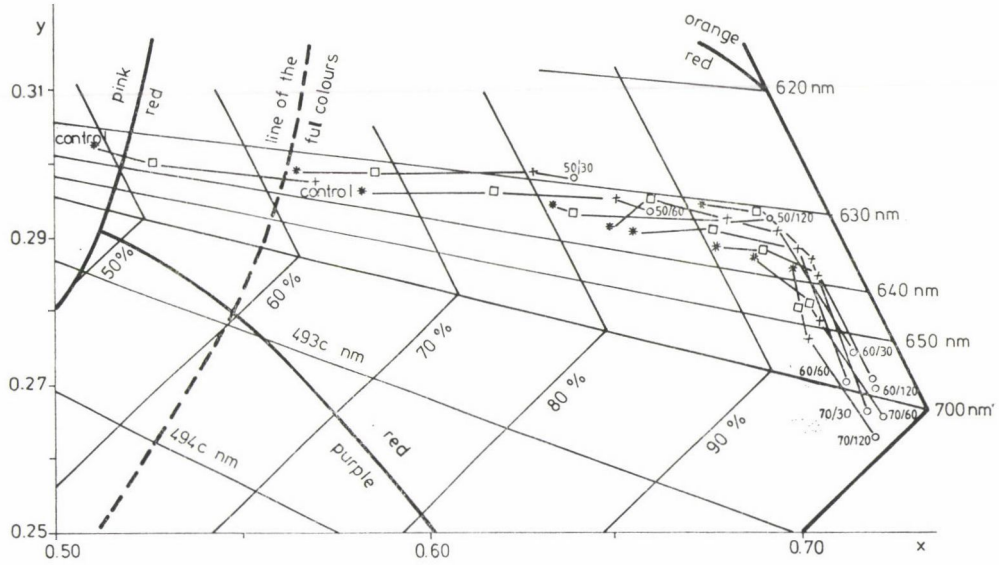


Fig. 4. Trends of the shift of colour points ($n = 39$) of red wines obtained by heat treatment and stored for 1 year, displayed in the chromaticity diagram. ○ = must; + = new wine; □ = wine once drawn; * = wine stored for 1 year

the darkest red colours. This is the place of the colour points of red wines of the highest pigment content, obtained by heating technique (parameters of treatment: at 60 and 70 °C for 60 and 120 min). It is noticeable that the colour points of the great variety of red wines, originating from different places of cultivation and of different varieties, obtained by different technologies are placed very closely along the curve. (The correlation coefficient, calculated from the straight part of the curve between the white point and spectrum locus is 0.92.)

### 2.3. *Correlation between* Y *and* x *colour characteristics of red wines calculated by the tristimulus method*

In accordance with the theoretical requirements the $Y$ value, approaching the spectrum locus trends to zero. On the other hand, approximating the white point the value of $Y$ increases and extrapolated, trends to 100. Values of $x$ vary only between the white point ($x = 0.3100$) and the corner of the colour triangle ($x = 0.7400$). As it can be seen in Fig. 3 to increasing $x$ values belong decreasing $Y$ values. The curve formed by 74 points of measured data crosses truly the point of coordinates $x = 0.74$; $Y = 0.00$. A hyperbola was fitted to the curve using regression analysis reduced to linear regression. The correlation coefficient of the corresponding linear relationship is 0.93.

### *2.4. Changes of the tristimulus values during storage of red wines prepared by heating technology*

There was no substantial difference in the change of the tristimulus values of wines of different years (1977, 1978, 1979) or different varieties (Kékfrankos, Cabernet franc). Therefore the results given in Tables 1, 2 and 3

Table 1

*Average change of the tristimulus values of Kékfrankos and Cabernet franc wines, obtained by heating to 50 °C in the years 1977, 1978 and 1979, during one year storage*

| Sample | Treatment (°C per min) | Tristimulus values | | | | | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | ±s | $\bar{y}$ | ±s | $\bar{Y}$ | ±s |
| Must | | | | | | | |
| New wine | | 0.5604 | 0.054 | 0.2975 | 0.026 | 9.30 | 0.66 |
| Wine once drawn | control | 0.5278 | 0.057 | 0.3016 | 0.033 | 10.27 | 0.98 |
| One year old wine | | 0.5110 | 0.052 | 0.3037 | 0.017 | 11.42 | 0.89 |
| Must | | 0.6394 | 0.059 | 0.2984 | 0.031 | 4.85 | 0.50 |
| New wine | 50/30 | 0.6287 | 0.064 | 0.2994 | 0.030 | 5.13 | 0.47 |
| Wine once drawn | | 0.5860 | 0.057 | 0.2984 | 0.027 | 7.85 | 0.59 |
| One year old wine | | 0.5659 | 0.054 | 0.2995 | 0.031 | 8.13 | 0.86 |
| Must | | 0.6593 | 0.095 | 0.2934 | 0.036 | 2.14 | 0.30 |
| New wine | 50/60 | 0.6507 | 0.073 | 0.2954 | 0.034 | 3.08 | 0.32 |
| Wine once drawn | | 0.6183 | 0.060 | 0.2961 | 0.027 | 6.47 | 0.57 |
| One year old wine | | 0.5820 | 0.061 | 0.2967 | 0.028 | 6.53 | 0.64 |
| Must | | 0.6919 | 0.071 | 0.2934 | 0.032 | 2.21 | 0.21 |
| New wine | 50/120 | 0.6792 | 0.068 | 0.2921 | 0.027 | 1.83 | 0.15 |
| Wine once drawn | | 0.6387 | 0.060 | 0.2933 | 0.028 | 4.54 | 0.55 |
| One year old wine | | 0.6331 | 0.059 | 0.2945 | 0.030 | 5.37 | 0.51 |

Table 2

*Average change of the tristimulus values of Kékfrankos and Cabernet franc wines, obtained by heating at 60 °C in the years 1977, 1978 and 1979, during one year storage*

| Sample | Treatment (°C per min) | Tristimulus values | | | | | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | ±s | $\bar{y}$ | ±s | $\bar{Y}$ | ±s |
| Must | | 0.7143 | 0.068 | 0.2746 | 0.026 | 1.78 | 0.22 |
| New wine | 60/30 | 0.6939 | 0.067 | 0.2916 | 0.028 | 1.97 | 0.20 |
| Wine once drawn | | 0.6605 | 0.065 | 0.2951 | 0.028 | 4.25 | 0.35 |
| One year old wine | | 0.6487 | 0.060 | 0.2919 | 0.027 | 4.91 | 0.35 |
| Must | | 0.7116 | 0.120 | 0.2707 | 0.032 | 1.90 | 0.22 |
| New wine | 60/60 | 0.6993 | 0.083 | 0.2884 | 0.034 | 2.08 | 0.20 |
| Wine once drawn | | 0.6767 | 0.090 | 0.2918 | 0.033 | 3.82 | 0.28 |
| One year old wine | | 0.6551 | 0.045 | 0.2918 | 0.026 | 4.03 | 0.23 |
| Must | | 0.7204 | 0.081 | 0.2698 | 0.030 | 1.86 | 0.10 |
| New wine | 60/120 | 0.7053 | 0.074 | 0.2857 | 0.027 | 1.92 | 0.22 |
| Wine once drawn | | 0.6907 | 0.053 | 0.2886 | 0.025 | 3.87 | 0.23 |
| One year old wine | | 0.6765 | 0.071 | 0.2882 | 0.022 | 3.51 | 0.33 |

Table 3

*Average change of the tristimulus values of Kékfrankos and Cabernet franc wines, obtained by heating to 70 °C in the years 1977, 1978 and 1979, during one year storage*

| Sample | Treatment (°C per min) | Tristimulus values | | | | | |
|---|---|---|---|---|---|---|---|
| | | $\bar{x}$ | ±s | $\bar{y}$ | ±s | $\bar{Y}$ | ±s |
| Must | 70/30 | 0.7171 | 0.080 | 0.2665 | 0.031 | 1.62 | 0.13 |
| New wine | | 0.7023 | 0.076 | 0.2874 | 0.023 | 2.61 | 0.31 |
| Wine once drawn | | 0.6882 | 0.059 | 0.2933 | 0.021 | 3.07 | 0.23 |
| One year old wine | | 0.6731 | 0.049 | 0.2944 | 0.033 | 2.03 | 0.18 |
| Must | 70/60 | 0.7220 | 0.083 | 0.2653 | 0.030 | 1.37 | 0.09 |
| New wine | | 0.7054 | 0.069 | 0.2782 | 0.019 | 1.22 | 0.13 |
| Wine once drawn | | 0.7020 | 0.061 | 0.2816 | 0.023 | 1.17 | 0.08 |
| One year old wine | | 0.6872 | 0.065 | 0.2871 | 0.031 | 2.95 | 0.37 |
| Must | 70/120 | 0.7209 | 0.061 | 0.2629 | 0.019 | 1.96 | 0.21 |
| New wine | | 0.7023 | 0.083 | 0.2768 | 0.026 | 2.58 | 0.26 |
| Wine once drawn | | 0.7007 | 0.065 | 0.2806 | 0.023 | 2.15 | 0.17 |
| One year old wine | | 0.6971 | 0.053 | 0.2860 | 0.021 | 2.45 | 0.23 |

are averages of the two varieties and three years for the must, the new wine, the wine once drawn and after one year storage. The changes of the colour points of red wines occurring during one year storage, are demonstrated in the red realm of the colour triangle enlarged in comparison to Figure 2 (Fig. 4).

## 3. Conclusions

Analysis of red wines fermented from different grape varieties, of different pigment contents and of different ripening times, grown at locations of optimal and weaker nature, permitted to acquire knowledge on the extreme and optimal values, thus the correlations established on the basis of the available measured data may be considered to be of general validity for Hungarian red wines.

From the tristimulus values measured during one year storage of red wines made by heating technology it was concluded that increasing maceration time and temperature have an effect on the location of the colour points of $x$ and $y$ coordinates in the colour triangle. With increasing maceration time and temperature the colour points of musts shifted to the right, toward the darker red colours (Fig. 4).

In the course of storage the monomer pigment content of the samples decreases and thus decreases the $A_{520}$ value, too (KAMPIS, 1980). In accordance to the correlation found between the values of $Y$ and $A_{520}$, as the $A_{520}$ values decrease, $Y$ values increase (Fig. 1). According to the correlation determined between $Y$ and $x$ values, to increasing $Y$ values belong decreasing $x$ values.

Thus, if during storage $A_{520}$ values of samples decrease, the $Y$ and $x$ values must show increasing and decreasing trends, respectively.

It can be seen in Fig. 4 that in agreement with expectations the $x$ values of separate samples truly show a decreasing tendency starting at the point determined in the must. The colour of the darkest musts with colour points in the corner of the colour triangle changed in accordance with the findings of ROBINSON and co-workers (1966a) during fermentation and storage: the colour points moved parallel with the spectrum locus while the hue shifted from purple towards orange-red. As soon as the colour points reached the region limited by the radial lines corresponding to wavelengths of 630–640 nm the trend changed. As it can be seen in Fig. 4 this occurred when the $x$ value reached the values of 0.69–0.70. This corresponds, as seen in Fig. 3, to an $Y$ value of 2. Hereafter, the colour points remained within this band and while their $x$ value decreased, they shifted towards the white point. Colour points belonging to musts of lighter colour from the very first in the realm limited by the radial lines correspond to 630–640 nm wavelengths. During storage their colour points, remaining within this band, shifted towards the white point. According to the colour theory the shift of the colour points along the radial lines belonging to constant wavelengths corresponds to continuous dilution of a pigment of fixed hue. Accordingly the spectral colours of the monomer and polymer pigments of red wines lighter than the value $Y = 2$ may not differ significantly. Otherwise the trend of shift of colour points during storage, i.e. the polymerization of monomer pigments (SOMERS, 1971) would result in a line far avoiding the white point on the chromaticity diagram. This is what happens in the case of musts and red wines darker than $Y = 2$ value, located in the corner of the colour triangle. The change of the hue from purple to orange-red corresponds to the transformation of a pigment into another one of a different hue, or to the decomposition of a purple pigment. The chemical identification of these pigments requires further study.

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# DETERMINATION OF THERMAL PROCESS SCHEDULE FOR CANNED MANGO, PAPAYA AND GUAVA PULPS

SIDDALINGU, B. SRINIVASAN, R. A. PADIVAL and S. RANGANNA

Central Food Technological Research Institute, Mysore-570 013. India

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Thermal process schedules for mango, papaya and guava pulps have been evolved on the basis of inactivation of pectinesterase (PE), the most heat resistant enzyme, present in these pulps. The process so evolved renders the canned product microbiologically safe.

Heat transfer into canned fruit pulps is by slow conduction. In products in which heat transfer is by conduction, the geometric centre is generally considered as the position of minimum lethality. This study shows that it is not so in fruit pulps filled hot into cans: the position shifts from or near the geometric centre towards the periphery of the can with an increase in the filling temperature from 73.9 °C to 87.8 °C. Hence, adequacy of processing depends upon achieving the desired $F$ value at the position of minimum lethality.

For Totapuri mango pulp (pH 4.0) filled into cans having 139.7 mm diameter and 181 mm height, the process time required to achieve $F_{97.2}^{10.3} = 1.7$ min ($F_{100}^{10.3} = 0.92$ min) at 97.2 °C is reduced from 63 min to 10 min when the filling temperature increases from 73.9 to 87.8 °C. Time lag between filling and processing further contributes to the lethality of the process. Thermal process schedule for Badami and Totapuri varieties of mango, papaya and guava pulps filled in 139.7×181 mm cans at different initial temperatures are also reported.

**Keywords:** Heat treatment of canned foods, enzyme inactivation, heat penetration

Thermal process schedule for acid products having pH between 4.0 and 4.5 is based on the destruction of sporeforming species other than *Clostridium botulinum*, especially *B. coagulans* among the anaerobes. In high-acid foods having pH $\leq$3.9, the spoilage is generally caused by non-sporulating bacteria like lactobacilli and leuconostoc, yeasts and moulds. Among the moulds, *Byssochlamys fulva* is more important. Heat resistance of lactobacilli, yeasts and moulds has been found to be lower than that of the heat resistant enzyme systems such as peroxidase, pectinesterase (PE) and polyphenoloxidase present in fruits. Unless inactivated, these cause undesirable changes (KAPLAN et al., 1949; NEBESKY et al., 1950; DASTUR et al., 1968). Thermal process schedule evolved on the basis of the enzyme inactivation rendered the canned product microbiologically safe (NANJUNDASWAMY et al., 1973; NATH & RANGANNA, 1977; 1980; 1981; 1983).

In the hot-fill-hold-cool procedure recommended by the NATIONAL CANNERS ASSOCIATION (NCA) (1970), fruit pulps below pH 4.0 are to be heated to 87.8 °C for 1 min, between pH 4.0 and 4.2 at 96.1 °C for 30 sec, filled into cans at a temperature of not less than 85 °C sealed and processed in steam

*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

or water at 87.8 °C for at least 2 min before cooling (RANGANNA, 1977). The above procedure is applicable provided the time lag between filling and sealing is not more than 1 min, the processing is continuous, and the can surface temperature is not less than 71.1 °C at the start of processing. In the conventional method of canning of fruit pulps in small canneries, the pulp is heated in a steam jacketted kettle, filled hot into 157.1×177.8 mm cans, sealed, processed in boiling water in batch type retorts and cooled. The time lag between sealing and processing of a batch of cans varies from 10 to 30 min when the temperature of the pulp decreases in the peripheral regions (NATH et al., 1983), and the thermal process time of 40–60 min commercially followed is empirical. In this paper, investigations carried out to determine the minimum thermal process schedule required for mango, papaya and guava pulps based on enzyme inactivation which would ensure the microbiological safety of the canned product are presented.

## 1. Materials and methods

### *1.1. Preparation of pulp*

Mango and papaya were washed, peeled, sliced, stones in mango and seeds of papaya removed, and the slices pulped. Guavas were lye-peeled in a hot 3% NaOH solution, washed, dipped in a 1% citric acid solution, cored, sliced and then pulped.

Pectinesterase activity was determined by the method of MACDONNEL and co-workers (1945) as modified by ROUSE and ATKINS (1952).

Consistency of the pulps measured using Brookfield synchroelectric viscometer, model LVT, with No. 4 spindle (Brookfield Engineering Laboratories, Inc. Stoughton, Mass., USA) is expressed in terms of power law functions (CHARM, 1963).

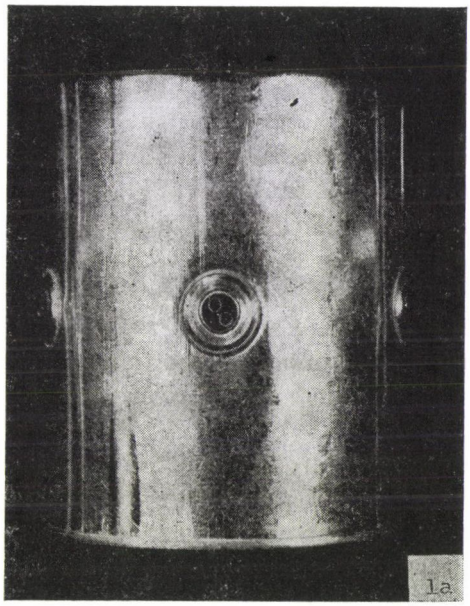
$$\tau = K(D)^n + C$$

where, $\tau$ = shear stress (dyn cm$^{-2}$), $K$ = fluid consistency coefficient (dyne s$^n$ cm$^{-2}$), $D$ = shear rate (s$^{-1}$), $n$ = flow behaviour index (dimensionless), and $C$ = yield stress (dyn cm$^{-2}$).

### *1.2. Heat penetration studies*

Heat penetration into the product packed into 139.7×181 mm cans was measured using Ecklund non-projecting, plug-in, needle-type thermocouples. Lead wires from the thermocouples were connected through a selector switch to a manually operated Leeds and Northrup potentiometer (Philadelphia, USA).

The mode of heat transfer into canned pulps is dominantly by conduction. In the preliminary experiments, it was observed that in the pulp filled hot (79.4–82.2 °C) into cans and processed for 40–60 min, the temperature increase at the geometric centre was negligible and during cooling, the temperature decreased very slowly. Consequently, when the filling temperature was 82.2 °C and above, the desired $F$ value at the geometric centre was achieved with little or no processing. Hence, 5 thermocouples were fixed on the centre of the body wall of the can (Fig. 1). Each thermocouple increased



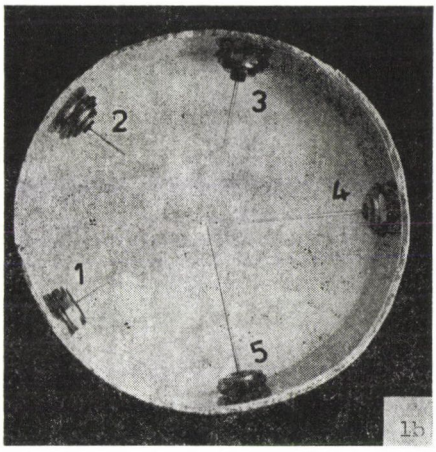




Fig. 1. Thermocouple positions in a 139.7×181 mm can used for determining the rate of heat penetration into canned pulps

Table 1

*Distance of the thermocouple tip from the inner periphery of the can*

| Thermocouple No. | Position No. | Distance (cm) |
|---|---|---|
| 1 | 1 | 2.2 |
| 2 | 2 | 2.5 |
| 3 | 3 | 3.8 |
| 4 | 4 | 5.2 |
| 5 | 5 | 6.9[a] |

[a] Geometric centre

progressively in length from the body wall of the can towards the centre, and the fifth reached the geometric centre. The distance of each thermocouple from the body wall of the can is given in Table 1.

The fill-in temperature of the pulps varied from 73.9 to 87.8 °C. Immediately after filling the pulp, the can was sealed, the thermocouples were connected to the potentiometer, and the canned pulp processed in boiling water (97.2 °C is the temperature at which water boils in Mysore which is situated at an altitude of 750 meters). The temperature changes at various positions in the canned product were measured at 1 min intervals during processing and cooling.

To measure the temperature changes and the lethality achieved during the time lag between filling and processing, the sealed cans were allowed to remain at room temperature for various time intervals to simulate the factory conditions before processing, and the temperature changes were measured during the hold-up time as above.

### *1.3. Process calculation*

Lethal rates at various temperatures during heating and cooling at different thermocouple positions were calculated using the expression:

$$L = \log^{-1} \frac{T_x - T}{z}$$

Where, $L$ is the lethal rate per minute at temperature $T$; $T_x$ is the reference temperature used to determine the $F$ value of the process, and $z$ is the temperature in °C required for the thermal inactivation time (TIT) curve to traverse through one log cycle.

The $F$ value achieved during heating and cooling at each of the five thermocouple positions for a specific time of processing was calculated by the

equal time interval procedure using the above formula (PATASHNIK, 1953) in a DCM programmable calculator. For each fill-in temperature, the cans were processed for different periods, and the $F$ values achieved at different thermocouple positions were calculated.

For any one filling temperature, the $F$ value vs. process time at each of the thermocouple positions were plotted on a graph paper (Fig. 3). Of the five

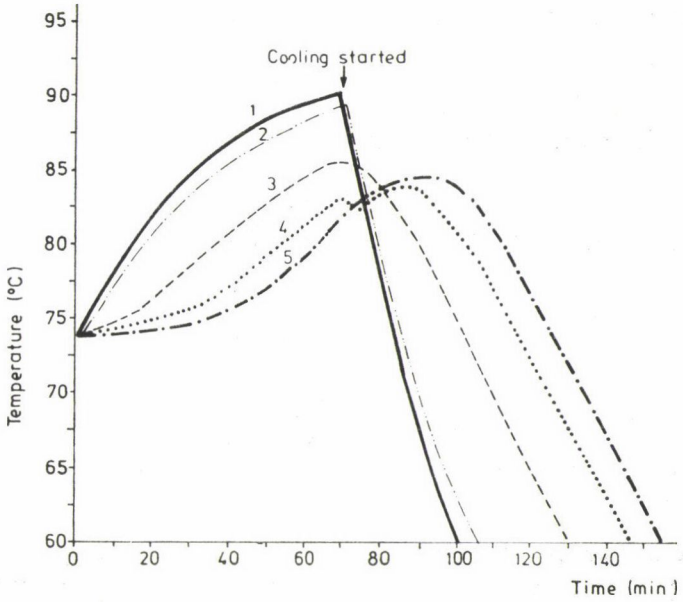



Fig. 2. Heating and cooling pattern of Totapuri mango pulp filled at 73.9 °C into 139.7×181 mm cans and processed for 70 min at 97.2 °C. 1, 2, 3, 4 and 5 refer to the thermocouple positions (see Table 1)

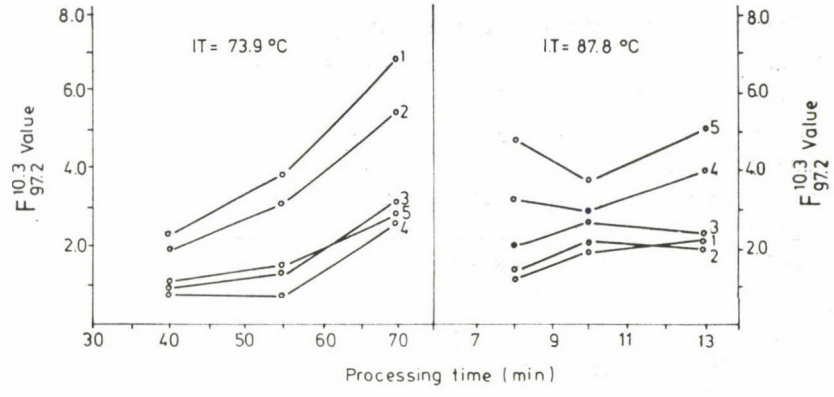



Fig. 3. Plot of $F$ value vs. process time at 97.2 °C. 1, 2, 3, 4 and 5 refer to the thermocouple positions (see Table 1). IT = Initial temperature

thermocouple positions, the position which achieved the lowest $F$ value during heating and cooling was the position of minimum lethality, and the time required to achieve the desired $F$ value at that position gave the process time.

## 2. Results and conclusions

### *2.1.* F *values for process calculation*

Table 2 gives the composition of mango, papaya and guava pulps. Table 3 gives the sterilization values which would inactivate and prevent regeneration of the PE, the most heat resistant enzyme in these fruits, and, in addition,

Table 2

*Total soluble solids (TSS), acidity, pH and pectinesterase (PE) activity in pulps and parameters for process calculation*

| | Totapuri mango | Papaya | Guava |
|---|---|---|---|
| TSS (%) | 11.50–18.00 | 10.50–12.00 | 10.00–13.50 |
| Acidity as anhydrous citric acid (%) | 0.21– 0.88 | 0.08– 0.24 | 0.22– 0.45 |
| pH value | 3.15– 4.30 | 4.20– 5.65 | 3.80– 4.15 |
| PE activity (PE Unit = $1 \times 10^4$ per cm$^3$) | 1.16– 5.83 | 2.67–15.00 | 7.80–19.90 |

render the canned products microbiologically safe with respect to microflora naturally present and the heat resistant test organism *Cl. pasteurianum* used in our studies (NATH & RANGANNA, 1980, 1981, 1983).

### *2.2. Heat transfer characteristics into canned pulp*

Mango, papaya and guava pulps are pseudoplastic fluids having yield stress (Table 4). The heat transfer into canned pulps was by slow conduction (Fig. 2). In Totapuri mango pulp, when the filling temperature was 73.9 °C, the temperature increased very slowly during heat processing in the regions near the geometric centre, i.e., at thermocouple positions 4 and 5. During cooling, the temperature decreased at positions 1, 2 and 3 but increased at positions 4 and 5 during the initial phase of cooling for 8–12 min before it decreased. The observations were almost similar when the filling temperature ranged from 76.7 to 87.8 °C except that at 85 and 87.8 °C, the temperature decreased instead of increasing at position 5 during heat processing. The heating and cooling patterns were similar in Alphonso mango and guava pulps. In papaya pulp, the temperature increase during the initial phase of cooling was not observed.

Table 3

F, D *and* z *values of pectinesterase and* Cl. pasteurianum *for process calculations*

| Particulars | Totapuri mango pulp = = syrup homogenate | Papaya pulp | Guava pulp = = syrup homogenate |
|---|---|---|---|
| TSS (%) | 20.0[b] | 10.5–12.00 | 20.0 |
| Acidity (%) | 0.41–0.43[b] | 0.42–0.46 | 0.21–0.26 |
| pH value | 3.6[b] | 4.0[e] | 4.0[e] |
| TIT[g] of PE | $F_{100}^{10.3} = 0.67$[c] | $F_{100}^{14.9} = 1.03$ | $F_{100}^{16.2} = 0.58$ |
| *D* value of PE | $D_{100}^{11.9} = 0.33$ | $D_{100}^{15.1} = 0.39$ | $D_{100}^{16.6} = 0.34$ |
| TDT[h] of *Cl. pasteurianum* | $F_{100}^{8.1} = 0.53$ | Does not grow | $F_{100}^{7.9} = 0.23$ |
| *F* value for process calculation[a] | $F_{100}^{10.3} = 0.76$[c,d] | $F_{100}^{14.9} = 1.3$ | $F_{100}^{16.2} = 1.23$ |
| *F* value at the processing temperature of 97.2 °C | 1.42 | 2.0 | 1.83 |
| Decimal reduction of enzyme equivalent to *F* value used for process calculation ($F/D$)[f] | 2.3 | 3.3 | 3.6 |

[a] Process equivalence recommended by NCA is $F_{100}^{8.9} = 0.18$ between pH 3.9 and 4.1 and $F_{100}^{8.9} = 0.44$ between 4.1 and 4.2 (NCA, 1968, 1970)

[b] In commercial samples of mango pulp from Alphonso, Totapuri, Neelum and other varieties, °Brix ranged from 14–20, acidity from 0.39–0.62% and pH from 3.6–4.1

[c] At pH 4.0, $F_{100}^{10.3} = 0.92$ is required

[d] Ripe Badami mango has very little PE activity; TIT of peroxidase is $F_{100}^{10} = 0.01$ (NANJUNDASWAMY et al., 1970)

[e] pH of the pulps generally canned

[f] $F/D$ in Totapuri mango e.g., 0.76/0.33 = 2.3

[g] Thermal inactivation time

[h] Thermal death time

Table 4

*Flow constants for pulps*

| | Temperature (°C) | $K$ (dyn $s^n$ $cm^{-2}$) | $n$ | $C$ (dyne $cm^{-2}$) |
|---|---|---|---|---|
| Totapuri mango | 28.5 | 28.0 | 0.535 | 157.0 |
| Papaya | 27.0 | 18.0 | 0.643 | 32.1 |
| Guava | 30.0 | 73.8 | 0.599 | 108.5 |

## *2.3. Calculation of processing time*

The temperature increase which occurs during the initial phase of cooling contributes a significant portion to the lethality of the slower heating zones in the container. Formula methods, as presently used for calculation of process time, are unable to include this lethality contributed (KOPELMAN et al., 1982). Hence the actual lethality achieved at different thermocouple positions during heating and cooling was calculated by the equal time interval method.

Totapuri mango pulp filled at 73.9 °C into 139.7×181 mm cans was processed for 40, 55 and 70 min and cooled. Fig. 3 shows the $F_{97.2}^{10.3}$ values achieved at different thermocouple positions during heat processing and cooling. When the pH of the pulp was 3.6, to achieve $F_{97.2}^{10.3} = 1.42$ ($F_{100}^{10.3} = 0.76$), the time required was 50.5, 56 and 60 min at positions 5, 3 and 4, respectively. At positions 1 and 2, much higher $F$ values were achieved during this period. Hence, position 4 which requires the maximum processing time to achieve the required $F$ value is the position of minimum lethality, and the processing time required is 60 min. Corresponding to the processing time of 60 min and $F_{97.2}^{10.3} = 1.42$ at position 4, the $F$ values achieved at positions 1, 2, 3 and 5 were 4.9, 3.83, 1.9 and 1.85, respectively. When the pH of the pulp is 4.0, $F_{97.2}^{10.3} = 1.7$ ($F_{100}^{10.3} = 0.92$) is required; the position of minimum lethality is 4,

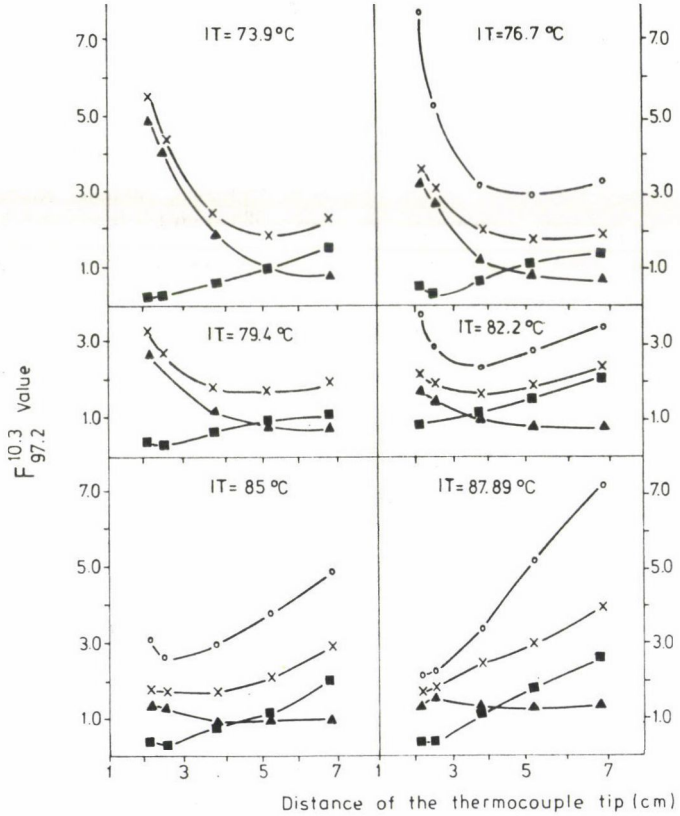



Fig. 4. $F$ value achieved at the end of calculated process time at different thermocouple positions during processing of Totapuri mango pulp in 139.7×181 mm cans

IT = Initial temperature
▲ = Heating
■ = Cooling
× = Heating + cooling
○ = Holding + heating + cooling

and the processing time required is 62.5 min. The $F$ values achieved corresponding to the processing time are 5.6, 4.45, 2.45 and 2.2 at positions 1, 2, 3 and 5, respectively. Hence, when the canned pulp is processed equal to the desired $F$ value at the position of minimum lethality, the $F$ value attained at other positions would be higher indicating that the entire can contents had been adequately processed.

When the filling temperature was 87.8 °C, to achieve $F_{97.2}^{10.3} = 1.42$, the time required at thermocouple position 1 was 8 min. By this time, the $F$ values achieved at thermocouple positions 2, 3, 4 and 5 were 2.1, 2.6, 2.9 and 3.7, respectively.

The above results show that the position of minimum lethality which was 4 when the filling temperature was 73.0 °C shifted to position 1 when the filling temperature was 87.8 °C, and the processing time at 97.2 °C was reduced from 60 to 8 min to achieve $F_{97.2}^{10.3} = 1.42$, and from 62.5 to 9.4 min to achieve $F_{97.2}^{10.3} = 1.7$. At other filling temperatures, the position of minimum lethality and the processing time required are given in Table 5. Figure 4 shows the $F$ values attained at other thermocouple positions corresponding to the $F$ value at the position of minimum lethality. The geometric centre (i.e. position 5) which is the point of slowest heating is not always the position of minimum lethality; the position shifts from the geometric centre towards the periphery with an increase in the filling temperature.

With increasing distance from periphery to the geometric centre (i.e. from thermocouple position 1 to 5), the lethality achieved during heating

Table 5

*Position of minimum lethality and thermal processing time at 97.2 °C for pulps packed in 139.7 × 181 mm cans*

| Initial temperature (°C) | Holding for 30 min[b] | Totapuri mango pulp | | | Badami mango pulp | | | Papaya pulp | | Guava pulp | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TC position[a] No. | Time (min) pH 3.6 | Time (min) pH 4.0 | TC position No. | Time (min) pH 3.6 | Time (min) pH 4.0 | TC position No. | Time (min) | TC position No. | Time (min) |
| 73.9 | — | 4 | 60 | 63 | 4 | 51 | 58 | 3 | 2.6 | 2 | 17 |
| | + | 3 | | | 4 | 54 | 62 | | | | |
| 76.7 | — | 4 | 37 | 44 | 4 | 39 | 46 | 2 | 18 | 1 | 14 |
| | + | 4 | | | 3 | 41 | 48 | | | | |
| 79.4 | — | 3 | 30 | 38 | 3 | 29 | 35 | 2 | 15 | 1 | 11 |
| | + | | 37 | 44 | 3 | 30 | 41 | | | | |
| 82.2 | — | 3 | 21 | 24 | 2 | 21 | 26 | 2 | 11 | 1 | 8 |
| | + | 3 | 30 | 38 | 3 | 24 | 28 | | | | |
| 85.0 | — | 2 | 14 | 16 | 1 | 13 | 16 | 1 | 7 | 1 | 6 |
| | + | 2 | 21 | 24 | 2 | 15 | 18 | | | | |
| 87.8 | — | 1 | 8 | 10 | 1 | 9 | 10 | 1 | 5 | 1 | 3 |
| | + | 1 | 14 | 16 | 1 | 11 | 13 | | | | |

[a] Position of minimum lethality with respect to thermocouple
[b] — without holding; + with holding. Processing time given in exclusive of holding time

Table 6

*Temperature changes during holding at different thermocouple positions and the lethality ($F_{97.2}^{10.3}$) achieved during holding and heat processing and cooling in Totapuri mango pulp canned in 139.7 × 181 mm cans*

| Filling temp. (°C) | Hold-up time (min) | Processing time (min) | | Thermocouple position 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|---|---|---|
| 76.7 | 20 | 60 | Actual initial temp., (°C) | 71.1 | 72.2 | 74.4 | 75.6 | 76.7 |
| | | | F value | | | | | |
| | | | – during hold-up | 0.08 | 0.10 | 0.15 | 0.18 | 0.20 |
| | | | – during heating & cooling | 7.85 | 5.11 | 2.99 | 2.73 | 3.19 |
| 82.2 | 30 | 37 | Actual initial temp., (°C) | 73.3 | 74.4 | 77.8 | 80.6 | 82.2 |
| | | | F value | | | | | |
| | | | – during hold-up | 0.26 | 0.31 | 0.55 | 0.85 | 1.12 |
| | | | – during heating & cooling | 3.56 | 2.48 | 1.82 | 2.01 | 2.40 |
| 85.0 | 30 | 30 | Actual initial temp., (°C) | 75.0 | 76.7 | 80.6 | 82.8 | 84.4 |
| | | | F value | | | | | |
| | | | – during hold-up | 0.45 | 0.56 | 0.98 | 1.41 | 1.95 |
| | | | – during heating & cooling | 2.77 | 2.03 | 1.95 | 2.44 | 3.02 |

decreases and vice versa during cooling (Fig. 4). This is due to heating and cooling by conduction.

The lethality achieved during hold-up time between sealing and processing of the hot-filled cans further contributes to lethality; the extent of contribution increases with increasing filling temperature, thermocouple position from 1 to 5, and hold-up time (Table 6). In addition to this, the pulp, if heated in a steam-jacketed kettle to the filling temperature, achieves some lethality. These have not been included in the processing time evolved at different temperatures as they may vary from factory to factory. They contribute to the additional safety of the processing time evolved.

### *2.4. Badami mango, papaya and guava pulps*

The processing time for Badami mango, papaya and guava pulp were similarly evolved for different filling temperatures with or without holding time. In Badami mango pulp, heating and cooling characteristics were similar to that of Totapuri mango pulp (Table 5). In papaya pulp, the position of minimum lethality was 3 at 73.9 °C, 2 at 76.7, 79.4 and 82.2 °C, and 1 at 85 °C and 87.8 °C; in guava pulp, the position was 2 at 73.9 °C and 1 at all other filling temperatures (Table 5). The variations observed with respect to papaya and guava pulps are attributed to comparatively lower viscosities of these pulps (Table 4). As in mango pulp, in guava and papaya pulps also, when the desired $F$ value is achieved at the position of minimum lethality, much higher $F$ values are achieved at other positions, indicating that the entire

contents of the can have been adequately processed. The process times calculated for 139.7 × 181 mm cans are given in Table 5.

Commercially packed pulps have a pH generally less than 4.0. If the pulp is heated to a temperature of 90.6 °C or more, and filled into cans at a temperature not less than 85 °C, mere holding the sealed cans for 5 min at room temperature or processing in boiling water for 3–5 min before cooling is adequate to render the canned product microbiologically safe. The process developed would result in better initial quality, increased productivity, and saving of energy. According to a report received from a factory, adoption of high temperature filling, holding and cooling for mango pulp which eliminated in-can processing as recommended by this study saved 10 litres of fuel oil per ton of mango pulp processed, increased the output, and reduced the spoilage in the canned product to as low as 0.01 %.

### *Nomenclature*

$F$, $F_T^z$ — Time in minutes required to inactivate the enzyme or destroy the organisms with a known $z$ at temperature $T$. In relation to processing, the symbols denote the sterilization value of the process

$L$ — Lethal rate per minute at product temperature $T$

$C$ — Yield stress (dyne $cm^{-2}$)

$IT$ — Initial temperature (°C)

$K$ — Fluid consistency coefficient (dyne $s^n$ $cm^{-2}$)

$n$ — Flow behaviour index (dimensionless)

$D$ — Shear rate ($s^{-1}$)

$T_x$ — Reference temperature used to determine the sterilization value ($F$) of the process

TIT — Thermal inactivation time of the enzyme (min)

TDT — Thermal death time of the organism (min)

$z$ — Temperature (°C) required for the TIT or the TDT curve to traverse one log cycle

$\tau$ — Shear stress (dyne $cm^{-2}$)

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# EFFECTS OF IRRADIATION AND FUMIGATION ON THE ANTIOXIDATIVE PROPERTIES OF SOME SPICES

D. P. KURUPPU[a,c], K. SCHMIDT[a,d], D. Is. LANGERAK[b,e], M. D. A. VAN DUREN[b] and J. FARKAS[a,d]

[a] International Facility for Food Irradiation Technology, P.O.Box 230, 6700 AE Wageningen. The Netherlands
[b] Research Institute ITAL, P.O.Box 48, 6700 AA Wageningen. The Netherlands

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The effects of gamma irradiation (5.6 kGy) and ethylene oxide fumigation (500 $g \cdot m^{-3}$ at room temperature, overnight) on the antioxidative activity of marjoram, nutmeg, paprika and black pepper were investigated. Sunflower oil in-water (1 : 1) emulsion (dark) at 30 °C, lard (dark) at 40 °C, lard (illuminated) at 50 °C and lard (dark) at 50 °C were the substrates utilized for the investigation. Oxidation of the substrates in the presence of 0.2% (by weight) of spices were followed by the determination of peroxide value (PO) and free fatty acid value (FFA). No significant effect of irradiation on the antioxidant activities of spices was revealed. Fumigated marjoram tended to be less antioxidative in lard kept in dark storage at 60 °C than the non-treated or irradiated spice. Marjoram and nutmeg lost their antioxidant properties in the presence of light. This loss, however, was not influenced by the irradiation or fumigation treatments.

**Keywords:** Antioxidative properties of spices, irradiation treatment, fumigation of spices

Spices and herbs are usually highly contaminated mainly by sporeforming aerobic bacteria. The total viable counts may reach $10^7\ g^{-1}$ level, whereas the mould counts vary between $10^2$–$10^6 g^{-1}$, that means that the addition of only 0.1–1% spice to various commodities can cause considerable contamination (FARKAS, 1983). At present, the most commonly used method for spice decontamination is the fumigation mainly with ethylene oxide. However, the use of fumigants has some serious disadvantages. The most critical one is the potential health hazard caused by the residual ethylene oxide absorptively bound, and ethylene halohydrins formed in the spices (EMBREE et al., 1977; KUČEROVA & ZHURKOV, 1977). Furthermore, the ethylene oxide treatment is not effective enough if the moisture content of the spice is low, the process itself is time-consuming, difficult to automate and cannot be made continuous. Because of these drawbacks, there is a growing interest in decontamination by

Present address:

[c] Atomic Energy Authority, 124, Barnes Place, Colombo 7. Sri Lanka
[d] Central Food Research Institute, P.O.Box 76, Budapest, 1525. Hungary
[e] State Institute for Quality Control of Agricultural Products, P.O.Box 230, 6700 AE Wageningen. The Netherlands

*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

ionizing radiations. Usually radiation doses of 3 to 7 kGy satisfactorily reduce the microbial counts (VAJDI & PEREIRA, 1973; INAL et al., 1975).

Besides the special flavour and spicing power, almost every spice has another important characteristic, the antioxidative effect (CHIPAULT et al., 1952, 1955). This may be due to several antioxidative compounds (flavonoids, cinnamic acid, hydroxybenzoic acid, etc.) or might be attributed to enzyme action (HERMANN, 1981; ERIKSSON, 1982). According to the literature survey, the effect of irradiation and fumigation on the antioxidative properties of the spices has not been investigated yet. Therefore we decided to study this question with the help of lard and oil-in-water emulsion as substrates.

In our experiment the possible changes in the antioxidative effect of spices were studied by the determination of the reaction products formed during the autooxidation process. The primary oxidation products, the peroxides were measured by PO-value, and the free fatty acids, the end products of the oxidation and lipase action [amongst the spices studied especially the black pepper has a relatively high lipase activity (PRUTHI, 1980)] by FFA-value.

## 1. Materials and methods

### *1.1. Materials*

*1.1.1. Spices.* Black pepper, marjoram, nutmeg and paprika were chosen for our investigation because of the considerable differences in their antioxidative properties (CHIPAULT et al., 1962; HERMANN, 1981).

The spices were obtained from the firm Ten Doesschate (Wapenveld, The Netherlands) in October 1982. One third of the spices was fumigated with ethylene oxide, another third irradiated, whereas the remaining part was left untreated.

*1.1.2. Substrates.* The lard and the sunflower oil were obtained from local retail shops and were free of artificial antioxidants. The oil-in-water (1 : 1) emulsion was prepared in a dairy homogenizer at 0.1 MPa after addition of 1% Triton X-100 and 1% propylene glycol emulsifiers at the Netherlands Dairy Research Institute (NIZO), Ede. The spice content was 0.2% in both media.

### *1.2. Methods*

*1.2.1. Irradiation.* The irradiation was carried out in the Pilot Plant for Food Irradiation, Wageningen, with a $^{60}$Co-source of 1.18 PBq with a dose rate of 2.14 kGy per hour. The average absorbed dose was 5.6 kGy.

*1.2.2. Fumigation.* The fumigation was carried out at the Ten Doesschate Company with T-gas (90% ethylene oxide, 10% carbon dioxide) at room temperature overnight. The fumigant concentration was 500 g $m^{-3}$.

Table 1

*Storage conditions of the spices and the analytical determinations carried out during storage*

| Storage medium | Spice | Temperature (°C) | Illumination | Duration of storage | Sampling | Analytical determination |
|---|---|---|---|---|---|---|
| Lard | Paprika<br>Black pepper<br>Marjoram<br>Nutmeg | 50 | dark | 14 weeks | every second week | PO-value<br>FFA-value |
| Lard | Marjoram | 50 | illuminated | 14 weeks | every second week | PO-value<br>FFA-value |
| Sunflower Oil-water emulsion | Paprika<br>Black pepper<br>Marjoram<br>Nutmeg | 30 | dark | 12 weeks | every second week | PO-value<br>FFA-value<br>Oil content |
| Lard | Marjoram | 60 | dark | 24 days | every day | PO-value<br>FFA-value |

*1.2.3. Storage.* The storage conditions and the analytical examinations carried out are listed in Table 1. The samples (20 g of lard and 30 g of emulsion) were stored in glass Petri dishes of 8.0 cm diameter. For the illumination FTD 18 W/33 type day light fluorescent tubes were used. The average distance between the lamps and the Petri dishes was 12 cm.

*1.2.4. PO-value.* Before the determination of the PO-value, the oil was extracted from the emulsion (the water content of the reaction mixture considerably influences the PO-value obtained) by shaking with chloroform and saturated NaCl solution. After separation the chloroform layer was dried by anhydrous $Na_2SO_4$. For the estimation of PO-value LEA's method (1952) was used.

*1.2.5. FFA-content.* The FFA-content was determined according to the official Dutch method (ANON., 1976). The 5–10 g fat or emulsion were weighed into an Erlenmeyer flask, 30 $cm^3$ neutralised ethanol and few drops of phenolphtalein indicator were added, and the mixture was warmed to promote dissolving. The solution was titrated with NaOH solution until the pink colour was stable for at least 20 seconds. The FFA-content was calculated as follows:

$$\text{FFA-value} = \frac{0.01\ N\ \text{NaOH}\ (\text{cm}^3) \times 0.282}{\text{mass of the sample (g)}}\ \%\ \text{of oleic acid in the sample.}$$

*1.2.6. Determination of the oil content in the emulsion.* The determination of the oil content was necessary because of the desiccation during storage. Five g emulsion was weighed onto a watch glass and heated on a hot plate to constant weight. The oil content was calculated from the residue of the sample.

## 2. Results

### *2.1. Marjoram*

Changes of oxidation products during storage determined as PO- and FFA-values for lard kept in the dark at 50 °C, containing marjoram, are shown in Figs. 1 and 2.

Lard without spice (control) showed a gradual increase in PO-value with storage time giving a maximum at 6 weeks after which the PO-value decreased. Lard containing spices showed an initial induction phase where no considerable change of PO-value has taken place, followed by an oxidation phase showing gradual increase of the PO-value (Fig. 1). Similar patterns of PO changes were observed for all the spices investigated.

The FFA-value gave an initial induction phase for the control as well as for the lard containing marjoram. The fat containing spice had a longer induction phase (Fig. 2).

Changes in oxidation products in the oil-water emulsion kept in the dark at 30 °C occurred at a much lower rate than for the lard at 50 °C. Under

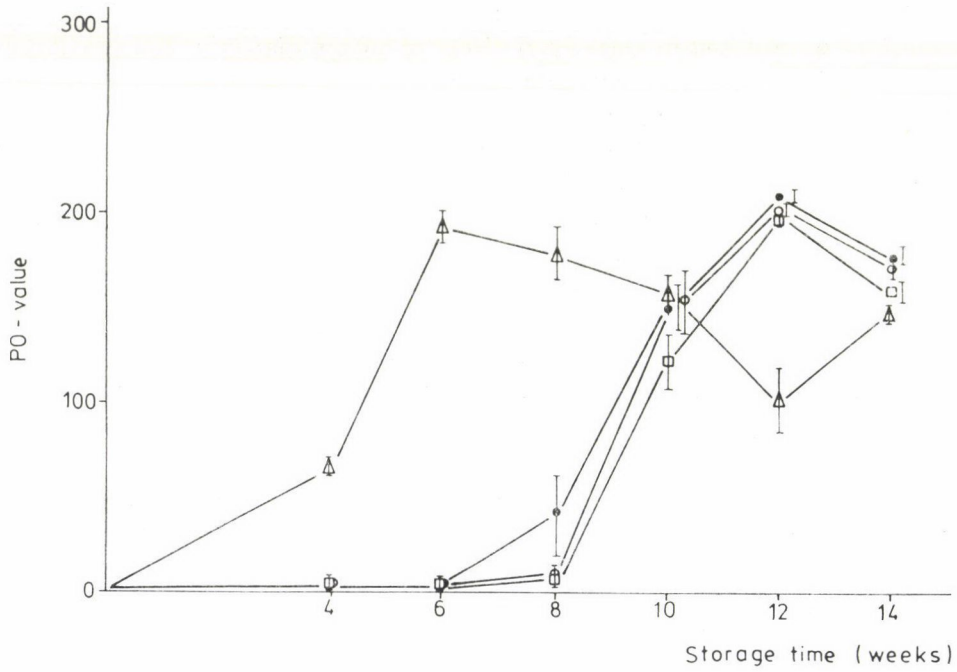


Fig. 1. Change in PO-value of control and lard containing differently treated marjoram during storage in the dark at 50 °C
△ = without spice (control)
□ = with non-treated spice
○ = with irradiated spice
● = with fumigated spice

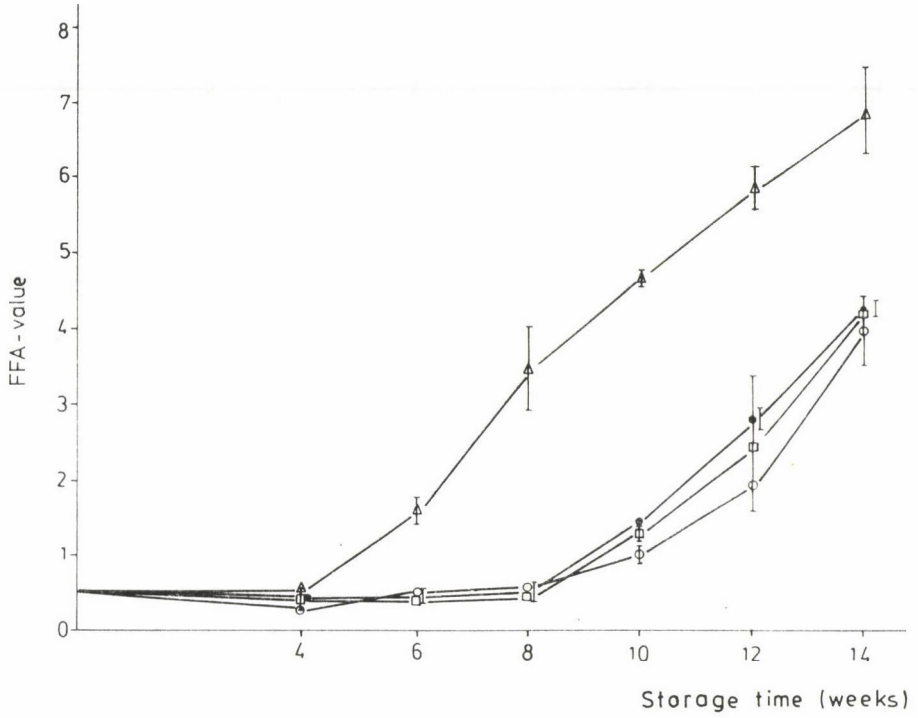


Fig. 2. Change in FFA-value of control and lard containing differently treated marjoram during storage in the dark at 50 °C
△ = without spice (control)
□ = with non-treated spice
○ = with irradiated spice
● = with fumigated spice

illuminated conditions, lard containing marjoram did not give an induction phase and the oxidation phase started from the initial stage of storage, almost similarly to the control.

Also lard kept in the dark at 60 °C gave almost a similar trend in variation of oxidation products as the lard at 50 °C, but at a higher rate.

PO- and FFA-values at specific storage times are summarized in Table 2. From the numerous data, following are shown in the tables: the PO- and FFA-values belonging to the 12th week in the case of the emulsion, to the 6th week in the case of the lard at 50 °C kept in the dark, to the 4th week for the illuminated lard and to the 1st week for the lard at 60 °C. These were chosen because of the differences in the rate of oxidation due to the different storage conditions.

The results show that for lipids kept in the dark PO- and FFA-values are lower for substrates containing marjoram than for the control. In the oil--in-water emulsion irradiation or fumigation did not influence the antioxidant

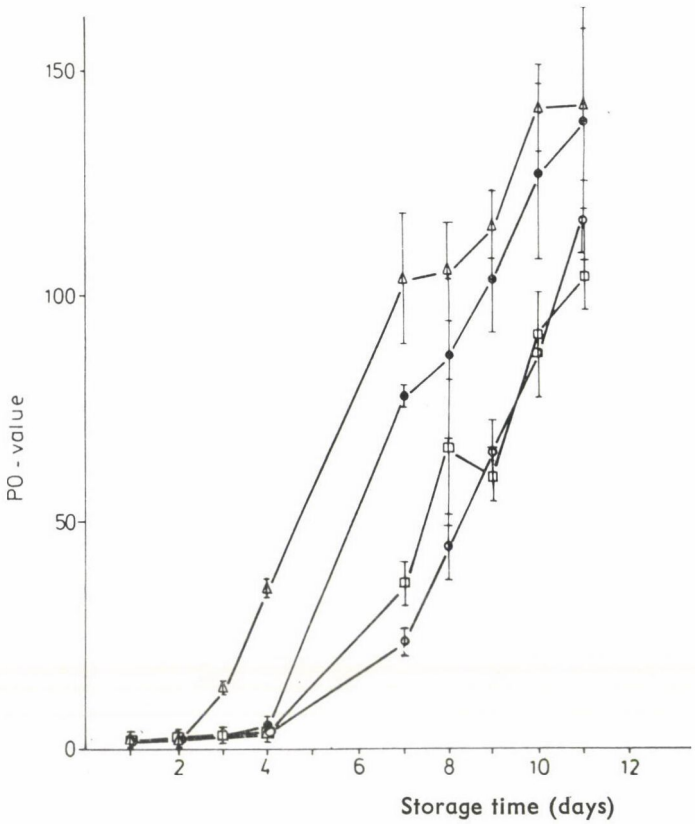


Fig. 3. Change in PO-value of control and lard containing differently treated marjoram during storage in the dark at 60 °C
△ = without spice (control)
□ = with non-treated spice
○ = with irradiated spice
● = with fumigated spice

property of marjoram. At 60 °C storage temperature, lard containing fumigated marjoram gave significantly higher PO-values than the fat containing non-heated or irradiated marjoram. Figure 3 shows that fumigated marjoram tended to be less antioxidative during the entire oxidation period under the experimental conditions than the non-treated or irradiated spice.

Under illuminated conditions marjoram lost its ability to act as an antioxidant in the lard. This loss, however, was not influenced by the irradiation or fumigation treatments.

### *2.2. Nutmeg*

PO- and FFA-values for the substrates containing nutmeg are collected in Table 3.

Table 2

*PO- and FFA-values of control (without spice) and substrates containing differently irradiated marjoram*

| | Peroxide value | | | | | | | | Free fatty acid value | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Lard (illuminated) at 50 °C for 4 weeks | | Lard (dark) at 60 °C for 1 week | | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Lard (illuminated) at 50 °C for 4 wekes | | Lard (dark) at 60 °C for 1 week | |
| | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s |
| Without spice | 317.9 | 95.7 | 192.4 | 8.8 | 154.0 | 21.1 | 104.3 | 15.1 | 1.1 | 0.2 | 1.6 | 0.2 | 2.0 | 0.4 | 0.6 | 0.0 |
| With non-treated spice | 65.2 | 5.7 | 2.8 | 0.2 | 170.7 | 37.5 | 36.8 | 5.4 | 0.2 | 0.0 | 0.5 | 0.2 | 1.4 | 0.0 | 0.5 | 0.0 |
| With irradiated spice | 79.1 | 23.5 | 2.6 | 0.2 | 168.3 | 9.0 | 24.2 | 2.8 | 0.2 | 0.0 | 0.4 | 0.0 | 1.5 | 0.0 | 0.5 | 0.0 |
| With fumigated spice | 69.0 | 0.2 | 3.5 | 0.9 | 191.9 | 13.7 | 78.8* | 2.1 | 0.2 | 0.0 | 0.5 | 0.0 | 1.9 | 0.5 | 0.5 | 0.0 |

* Indicates statistically significant difference at P = 5% probability level from the substrate containing non-treated or irradiated spice
Number of measurements (n) = 3

Table 3

*PO- and FFA-values of control (without spice) and substrates containing differently treated nutmeg*

| | Peroxide value | | | | | | Free fatty acid value | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Lard (illuminated) at 50 °C for 4 weeks | | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Lard(illuminated) at 50 °C for 4 weeks | |
| | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s |
| Without spice | 317.9 | 95.7 | 192.4 | 8.8 | 154.0 | 24.4 | 1.1 | 0.2 | 1.6 | 0.2 | 2.0 | 0.3 |
| With non-treated spice | 48.6 | 3.1 | 59.9 | 22.1 | 130.2 | 19.7 | 0.4 | 0.0 | 0.7 | 0.0 | 1.6 | 0.5 |
| With irradiated spice | 47.4 | 5.5 | 91.3 | 12.0 | 134.7 | 3.4 | 0.3 | 0.2 | 0.8 | 0.0 | 1.6 | 0.5 |
| With fumigated spice | 40.8 | 2.6 | 88.6 | 24.2 | 1 49.5 | 1.7 | 0.3 | 0.0 | 0.8 | 0.0 | 1.5 | 0.3 |

Number of measurements (n) = 3

It can be seen from the lower values of PO and FFA in the substrates containing nutmeg, in comparison with the control, that nutmeg had an antioxidant effect. At the same time, no significant difference of PO- and FFA-values was obtained between the substrates containing nutmeg treated in different ways, revealing that irradiation or fumigation had no effect on the antioxidant activity. Under illuminated conditions loss of antioxidant activity of nutmeg was shown by the absence of significant difference in PO- and FFA-values between the fat containing nutmeg and the control. Irradiation or fumigation also did not show any effect on the loss of antioxidant activity.

### *2.3. Paprika*

PO- and FFA-values of the substrates containing paprika are collected in Table 4.

Lower PO- and FFA-values of the emulsion containing paprika substantiated, in contrast to the control, the antioxidant property of paprika in

Table 4

*PO- and FFA-values of control (without spice) and substrates containing differently treated paprika*

| | Peroxide value | | | | Free fatty acid value | | | |
|---|---|---|---|---|---|---|---|---|
| | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | |
| | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s |
| Without spice | 317.9 | 95.7 | 192.4 | 8.8 | 1.1 | 0.2 | 1.6 | 0.2 |
| With non-treated spice | 51.7 | 11.6 | 165.9 | 5.1 | 0.5 | 0.3 | 1.0 | 0.0 |
| With irradiated spice | 40.5 | 6.4 | 171.9 | 19.5 | 0.2 | 0.0 | 1.0 | 0.2 |
| With fumigated spice | 53.8 | 12.3 | 149.4 | 17.0 | 0.5 | 0.2 | 1.0 | 0.0 |

Number of measurements (n) = 3

the emulsion, and the absence of a significant difference of PO- and FFA-values between the treatments also revealed that irradiation or fumigation did not have any effect on the antioxidant property of paprika. In lard kept in the dark at 50 °C, PO-values were lower for the fat containing paprika than for the control, even though the values did not show a statistically significant difference. However, FFA-values were significantly lower in the fat containing paprika than in the control. There was no significant difference between the activities of differently treated paprika.

### *2.4. Black pepper*

PO- and FFA-values of the substrates containing pepper are given in Table 5.

Table 5

*PO- and FFA-values of control (without spice) and substrates containing differently treated black pepper*

| | Peroxide value | | | | Free fatty acid value | | | |
|---|---|---|---|---|---|---|---|---|
| | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | | Oil-in-water emulsion (dark) at 30 °C for 12 weeks | | Lard (dark) at 50 °C for 6 weeks | |
| | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s | $\bar{x}$ | ±s |
| Without spice | 317.9 | 95.7 | 192.4 | 15.3 | 1.1 | 0.2 | 1.6 | 0.2 |
| With non-treated spice | 55.6 | 1.9 | 61.5 | 14.6 | 0.7 | 0.5 | 3.8 | 0.3 |
| With irradiated spice | 88.0 | 20.0 | 38.6 | 10.8 | 1.9 | 0.9 | 3.9 | 0.5 |
| With fumigated spice | 89.0 | 13.1 | 40.8 | 4.3 | 1.0 | 0.3 | 4.0 | 0.3 |

Number of measurements (n) = 3

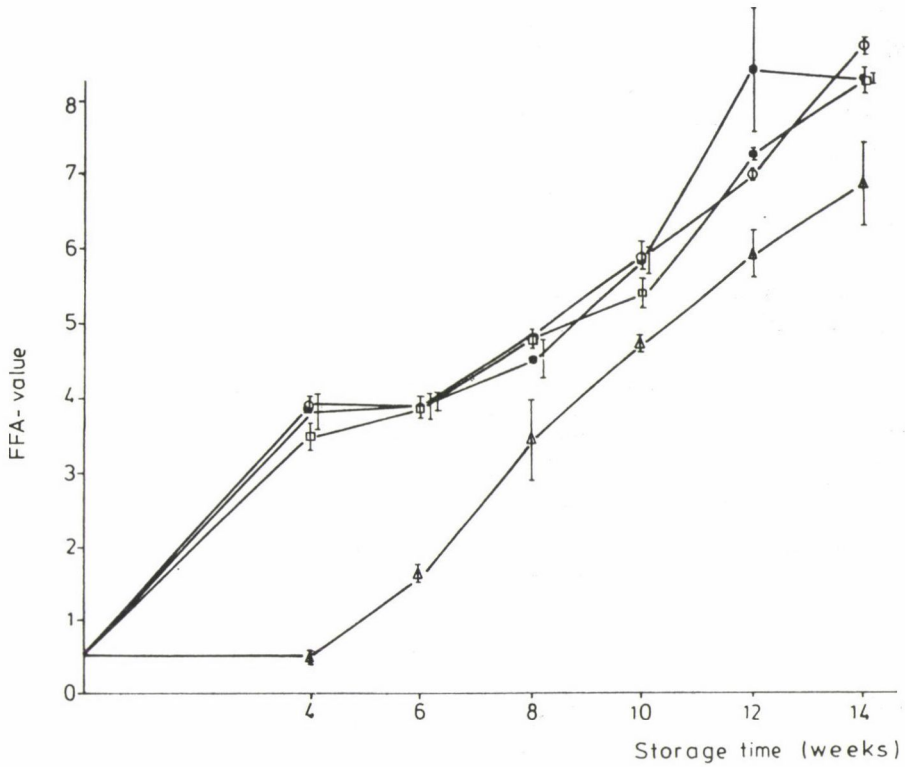


Fig. 4. Change in FFA-value of control and lard containing differently treated black pepper during storage in the dark at 50 °C
△ = without spice (control)
□ = with non-treated spice
○ = with irradiated spice
● = with fumigated spice

From the lower PO-values found in the substrates containing pepper, it can be seen that, compared to the control, pepper exhibited antioxidant activity in both substrates. Irradiation or fumigation did not affect the antioxidant property of pepper.

FFA-values of lard containing pepper gave significantly higher values than the control (Fig. 4). This behaviour is attributed to the lipase activity of pepper (PRUTHI, 1980; HALBERT et al., 1966), which produces fatty acids by enzymic hydrolysis of fat. Figure 4 clearly shows the formation of free fatty acids by enzymic hydrolysis at the initial stage of storage followed by oxidative formation of free acids in the oxidation phase. Irradiation or fumigation did not influence the lipolytic activity of pepper.

## 3. Conclusions

Marjoram, nutmeg, paprika and black pepper exhibited antioxidant activity retarding the oxidation process of oil-in-water emulsion at 30 °C and lard at 50 °C and 60 °C in the dark.

Marjoram and nutmeg which were investigated in lard substrate under illuminated conditions lost their antioxidant properties in the presence of light, which is in agreement with earlier findings reported (HERMANN, 1981).

Irradiation did not show any significant influence on the antioxidant activities of the above mentioned spices in the emulsion at 30 °C and lard kept in the dark at 50 °C and 60 °C. Fumigated marjoram tended to be less antioxidative in lard kept in the dark at 60 °C than the non-treated or irradiated spice.

*

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# COMPARATIVE EVALUATION OF TRIAL SHIPMENTS OF FUMIGATED AND RADIATION DISINFESTED DATES FROM IRAQ

M. S. H. AHMED[a], A. A. HAMEED[a], A. A. KADHUM[a], S. R. ALI[a], J. FARKAS[b,e], D. IS. LANGERAK[c,d] and M. D. A. VAN DUREN[c]

[a] Faculty of Agriculture and Biology, Nuclear Research Centre, P.O. Box 765, Baghdad. Iraq
[b] International Facility for Food Irradiation Technology, c/o RIKILT, P.O. Box 230, 6700 AE Wageningen. The Netherlands
[c] Research Institute ITAL, P.O. Box 48, 6700 AA Wageningen. The Netherlands

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Commercial dried dates of the Zahdi variety, with a "natural infestation" of 2.7 insects per 100 fruits, packed in 250 g "window carton lunch boxes" and wrapped in cellophane, as well as artificially infested dates (deliberately infested with *Ephestia cautella* and *Oryzaephilus surinamensis* to an infestation of 114 insects per 100 fruits) packed in lunch boxes and wrapped in cellophane or polyethylene foil were disinfested by using either the commercial methyl bromide fumigation or irradiation with 0.70 kGy gamma rays from a $^{60}$Co source in Iraq. Forty packages each of the experimental batches were placed in standard carton boxes and sent by air to the International Facility for Food Irradiation Technology (IFFIT), Wageningen, The Netherlands, for comparative evaluation at five periods of storage ranging from 64 to 207 days. The results indicate that: irradiation with 0.7 kGy gamma rays is more effective for disinfesting dates than commercial methyl bromide fumigation; commercial cellophane wrapping is insufficient to prevent re-infestation; and properly sealed polyethylene wrapping is capable to prevent re-infestation under the storage conditions applied.

**Keywords:** Fumigation of dates, irradiation treatments of dates, disinfestation

Packed dried dates are exported throughout the world to more than 60 countries (HUSSAIN, 1974). The main problem encountered in date trade comes from infestation by stored-product insect pests, which regularly necessitates repeated fumigation with methyl bromide to comply with the various quarantine measures of different importing countries (CORNWELL, 1966). On the other hand, using any chemicals involves problems with residues and with unwanted effects (BRYNJOLFSSON, 1981), so that a physical treatment such as irradiation looks more effective and safer as an alternative to chemical disinfestation treatment of commercially packed dried dates (AHMED et al., 1984).

Present address:

[d] State Institute for Quality Control of Agricultural Products, P.O. Box 230, 6700 AE Wageningen. The Netherlands.
[e] Central Food Research Institute, P.O. Box 76, Budapest, 1525 Hungary

*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

The aim of the present experiments is to evaluate the trial shipments of packed dried dates exported to the Netherlands with the assistance of the International Facility for Food Irradiation Technology (IFFIT) to investigate

— the disinfestation efficiency of packed dates after treatment with methyl bromide or with 0.7 kGy gamma radiation,

— the capability of both commercial cellophane wrapping and polyethylene foil to prevent re-infestation of the treated dates by two insect species or any other penetrator or invader species (CLINE, 1978; CLINE & HIGHLAND, 1981; HIGHLAND & WILSON, 1981).

## 1. Materials and methods

### *1.1. Commercial dates*

Dates, Zahdi variety which amount to approximately 66% of the total production and comprise almost 80% of the annually exported dry dates in Iraq (HUSSAIN, 1974), were obtained in small packages destined for commercial purposes in collaboration with the Iraqi Dates Administration. The infestation rate was 6 insects/220 dates (2.7%) which is apparently acceptable to some importing countries if the insects are dead in a more or less similar situation as the concept of probit 9 security standard (BURDITT, 1982) where the survivors are usually fertile after fumigation contrary to the effect of gamma radiation which would yield absolutely sterile live adults, if any (GIDDINGS, 1983). Eighty of the small packages which are commonly called window carton lunch boxes, each holding 250 g of dried dates, were automatically sealed with cellophane film (CB) at the Baghdad Packing House (AHMED et al., 1982) and placed in two standard carton boxes (SCB). One of the SCBs was subjected to fumigation using ca. 0.5 kg of methyl bromide per ca. 28 $m^3$ for 24 h in an atmospheric chamber. The other 40 CBs were placed two at a time in a Gammacell-220 irradiation chamber with a dose rate of approximately 1.22 kGy $h^{-1}$. The dose was 0.70 kGy at the central field of the chamber with a $D_{max}/D_{min}$ of ca. 1.4 (AECL, 1966).

### *1.2. Artificially infested dates*

Deliberate infestation of dates, Zahdi variety of 1982 harvest, was accomplished according to the method mentioned by AHMED and co-workers (1982) with minor alterations. Forty kg of dates were put in four fiberglass cages (10 kg per cage, internal dimensions: $40 \times 40 \times 40$ cm). Then an almost uniform distribution of intentional infestation by all stages of *Ephestia cautella* and *Oryzaephilus surinamensis* was obtained by adding females and males of both species as illustrated in the following table:

| *Date of infestation* | *Number of insects added to each cage* |
|---|---|
| | *Ephestia cautella* adults (fig moths) |
| 25/10/82 | 7 females × 6 males |
| 26/10/82 | 6 females × 6 males |
| 31/10/82 | 15 females × 15 males |
| 7/11/82 | 15 females × 22 males |
| 20/11/82 | 15 females × 15 males |
| 20/11/82 | 1500 *Oryzaephilus surinamensis* adults without sexing (saw-toothed grain beetles) |

On 29 November 1982, a sample of 116 date fruits taken at random from the four above-mentioned cages was examined in which 168 insects were found (144.8%), i.e. 2086 insects per treatment. At this time 80 lunch boxes were filled with infested dates as indicated elsewhere (AHMED et al., 1982) and wrapped in cellophane (CBs). Then 40 CBs were put in one standard carton box (SCB) followed by treatment with methyl bromide as in case of commercial batches. While the other 40 CBs were treated with 0.7 kGy of gamma radiation as previously mentioned. Furthermore, 80 date-filled lunch boxes were similarly treated after wrapping in polyethylene foil (PB), instead of cellophane, by hand using a heat sealer to test the efficiency of polyethylene vs. cellophane in preventing insect reinfestation. One SCB filled with 40 PBs and a second one filled with CBs were treated with methyl bromide; the other 2 SCBs (40 CBs and 40 PBs) were treated with a dose of gamma radiation, as in the case of commercial dates as described under 1.1 above.

### *1.3. Transportation and storage*

After treatment the four SCBs of deliberately infested dates side by side with the 2 SCBs of commercial dates were transported by air to the International Facility for Food Irradiation Technology, Wageningen (The Netherlands) for storage and evaluation.

On arrival at IFFIT they were stored at 20 °C and ca. 60% R.H. On 15 January 1983 the SCBs were put in polyethylene bags to prevent escape of insects.

### *1.4. Inspection of samples*

Examinations of samples of 8 packages taken from each of the six SCBs were carried out 64, 93, 142, 178 and 207 days after treatment, respectively. The numbers of infested dates and the live insects per lunch box were recorded

Table 1

*Numbers of live insects found in eight "lunch boxes" of commercial dates*

| Date of inspection in 1983 | Days after disinfest. | Fumigated | | | Irradiated | | |
|---|---|---|---|---|---|---|---|
| | | Ephestia | Oryzae-philus | Total | Ephestia | Oryzae-philus | Total |
| February 2 | 64 | 2 | 2 | 4 | 0 | 4 | 4 |
| March 3 | 93 | 1 | 1 | 2 | 0 | 4 | 4 |
| April 21 | 142 | 1 | 30 | 31 | 0 | 37 | 37 |
| May 27 | 178 | 2 | 35 | 37 | 0 | 24 | 24 |
| June 25 | 207 | 0 | 92 | 92 | 0 | 265 | 265 |

Table 2

*Frequencies of numbers of live* Oryzaephilus *found in "lunch boxes" of commercial dates treated with methyl bromide or gamma radiation and examined at five intervals of storage ranging from 64 to 207 days*

| Treatment | Number of live insects per box | Storage time (day) | | | | | Storage time effect |
|---|---|---|---|---|---|---|---|
| | | 64 | 93 | 142 | 178 | 207 | |
| Fumigation | 0 — < 8 | 8 | 8 | 6 | 7 | 6 | |
| | 8 — <16 | | | 1 | | | |
| | 16 — <24 | | | 1 | | | |
| | 24 — <32 | | | | 1 | 2 | |
| | 32 — <40 | | | | | | |
| | 40 — <48 | | | | | | * |
| | 48 — <56 | | | | | | |
| | 56 — <64 | | | | | | |
| | 64 — <72 | | | | | | |
| | 72 — <80 | | | | | | |
| | 80 — <88 | | | | | | |
| | 88 — <96 | | | | | | |
| Irradiation | 0 — < 8 | 8 | 8 | 7 | 6 | 4 | |
| | 8 — <16 | | | | 2 | | |
| | 16 — <24 | | | | | | |
| | 24 — <32 | | | | | 1 | |
| | 32 — <40 | | | 1 | | | |
| | 40 — <48 | | | | | | ** |
| | 48 — <56 | | | | | | |
| | 56 — <64 | | | | | 1 | |
| | 64 — <72 | | | | | | |
| | 72 — <80 | | | | | 1 | |
| | 80 — <88 | | | | | | |
| | 88 — <96 | | | | | 1 | |
| Treatment differences | | Ø | Ø | Ø | Ø | Ø | |

Eight boxes were examined at each time for each treatment

Treatment differences (for the same day) were evaluated using the Wilcoxon (Mann-Whitney) two sample statistics (Weber, 1961). Storage time effects (for the same treatment) were investigated by calculating the Spearman rank correlation coefficient (Hilhorst, 1981)

Ø Non significant ($P > 0.05$)

* Significant ($P \leq 0.05$)

** Highly significant ($P \leq 0.01$)

*** Very highly significant ($P \leq 0.001$)

Table 3

*Numbers of insects found in the SCBs outside the lunch boxes of commercial dates*

| Date of inspection in 1983 | Days after disinfest. | Fumigation | | Irradiation | |
|---|---|---|---|---|---|
| | | Ephestia | Oryzae-philus | Ephestia | Oryzae-philus |
| February 2 | 64 | 0 | 1 | 0 | 0 |
| March 3 | 93 | 0 | 0 | 0 | 0 |
| April 21 | 142 | 0 | 0 | 0 | 0 |
| May 27 | 178 | 1 | 0 | 0 | 0 |
| June 25 | 207 | 0 | 4 | 0 | 6 |

Table 4

*Frequencies of percentage infestation of commercial dates packed in lunch boxes, treated with methyl bromide or gamma radiation and examined at five intervals of storage ranging from 64 to 207 days*

| Treatment | Percentage infested[a] fruits | Storage time (days) | | | | | Storage time effect |
|---|---|---|---|---|---|---|---|
| | | 64 | 93 | 142 | 178 | 207 | |
| Fumigation | 0 — < 7 | 5 | 2 | 3 | 4 | | |
| | 7 — <14 | 2 | 2 | | 3 | 3 | |
| | 14 — <21 | 1 | 1 | 5 | | 1 | |
| | 21 — <28 | | 3 | | | 1 | |
| | 28 — <35 | | | | | | |
| | 35 — <42 | | | | 1 | 2 | |
| | 42 — <49 | | | | | | * |
| | 49 — <56 | | | | | | |
| | 56 — <63 | | | | | | |
| | 63 — <70 | | | | | | |
| | 70 — <77 | | | | | | |
| | 77 — <84 | | | | | 1 | |
| Irradiation | 0 — < 7 | 8 | 7 | 6 | 4 | | |
| | 7 — <14 | | 1 | 1 | 3 | | |
| | 14 — <21 | | | | | 3 | |
| | 21 — <28 | | | 1 | | 1 | |
| | 28 — <35 | | | | 1 | | |
| | 35 — <42 | | | | | | *** |
| | 42 — <49 | | | | | | |
| | 49 — <56 | | | | | | |
| | 56 — <63 | | | | | 1 | |
| | 63 — <70 | | | | | 1 | |
| | 70 — <77 | | | | | 2 | |
| | 77 — <84 | | | | | | |
| Treatment differences | | ** | ** | Ø | Ø | Ø | |

For explanation and symbols see Table 2

to assess the killing effect brought about by both commercial fumigation and experimental gamma radiation as well as the occurrence of re-infestation. Dried fruits were considered to be infested if they contained any dead or live insect stage, or just insect excretion alone observable with the naked eye.

## 2. Results and discussion

### *2.1. Commercial dates*

Various stages of both *Ephestia* and *Oryzaephilus* were found alive in the fumigated lot, while only alive *Oryzaephilus* occurred in the irradiated lot. The numbers of live insects found in 8 boxes examined for five storage times are shown in Table 1.

The frequency-distribution of the number of live *Oryzaephilus* as a function of the storage time and the results of the statistical evaluation of treatment differences and storage-time effects, resp., are shown in Table 2.

The build-up of the *Oryzaephilus* population during storage both in the fumigated lot and the irradiated one was proven by the Spearman tests for rank correlation coefficient (HILHORST, 1981). No significant differences were found at either storage intervals between the population in fumigated boxes and that of irradiated ones, using the Wilcoxon (Mann-Whitney) two sample statistic (WEBER, 1961).

Table 3 shows the numbers of insects found in the SCBs outside the lunch boxes.

Frequency distribution of the percentage of infested dates and their statistical evaluation are given in Table 4.

The percentages of infested dates were significantly lower in irradiated packages than in fumigated ones stored for 64 or 93 days. At a longer storage time the percentage of infested dates increased both in irradiated and fumigated packages, and the treatment differences became non-significant.

The above data strongly suggest that such packages of commercial dates with cellophane wrapping are not satisfactory to prevent re-infestation for any of the storage periods mentioned, since live insects were found both in the fumigated and the irradiated batches (see Table 1). The present results confirm the earlier findings (AHMED et al., 1982) where such packages were stored for 25, 55 and 80 days, respectively, and packages stored for 25 days only were not reinfested.

### *2.2. Artificially infested dates*

Each figure presented in Table 5 shows the number of live insects found in 8 polyethylene- or cellophane-wrapped lunch boxes of artificially in-

Table 5

*Numbers of live insects found in eight "lunch boxes" of artificially infested dates*

| Date of inspection in 1983 | Days after disinfest. | Fumigated | | | | | | Irradiated | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cellophane | | | Polyethyene | | | Cellophane | | | Polyethyene | | |
| | | Ephestia | Oryzaephilus | Total | Ephestia | Oryzaephilus | Total | Ephestia | Oryzaephilus | Total | Ephestia | Oryzaephilus | Total |
| February 2 | 64 | 24 | 322 | 346 | 2 | 151 | 153 | 0 | 4 | 4 | 0 | 0 | 0 |
| March 3 | 93 | 3 | 590 | 593 | 1 | 659 | 660 | 0 | 2 | 2 | 1 | 3 | 4 |
| April 21 | 142 | — | — | — | — | — | — | 0 | 105 | 105 | 0 | 54 | 54 |
| May 27 | 178 | — | — | — | — | — | — | 0 | 297 | 297 | 1 | 0 | 1 |
| June 25 | 207 | — | — | — | — | — | — | 0 | 535 | 535 | 0 | 2 | 2 |

—: Not investigated

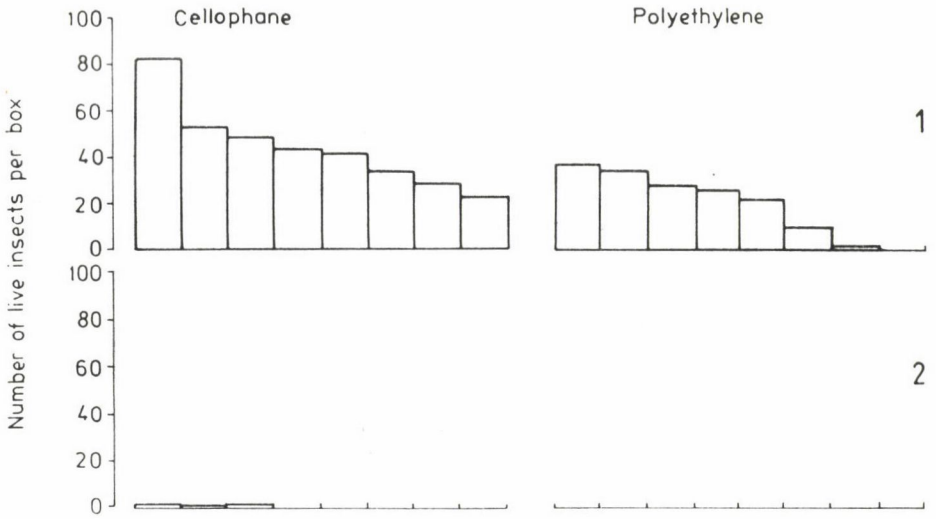


Fig. 1. Numbers of live insects per cellophane- or polyethylene-wrapped box 64 days after fumigation (1) or irradiation (2). Eight boxes were examined for each treatment

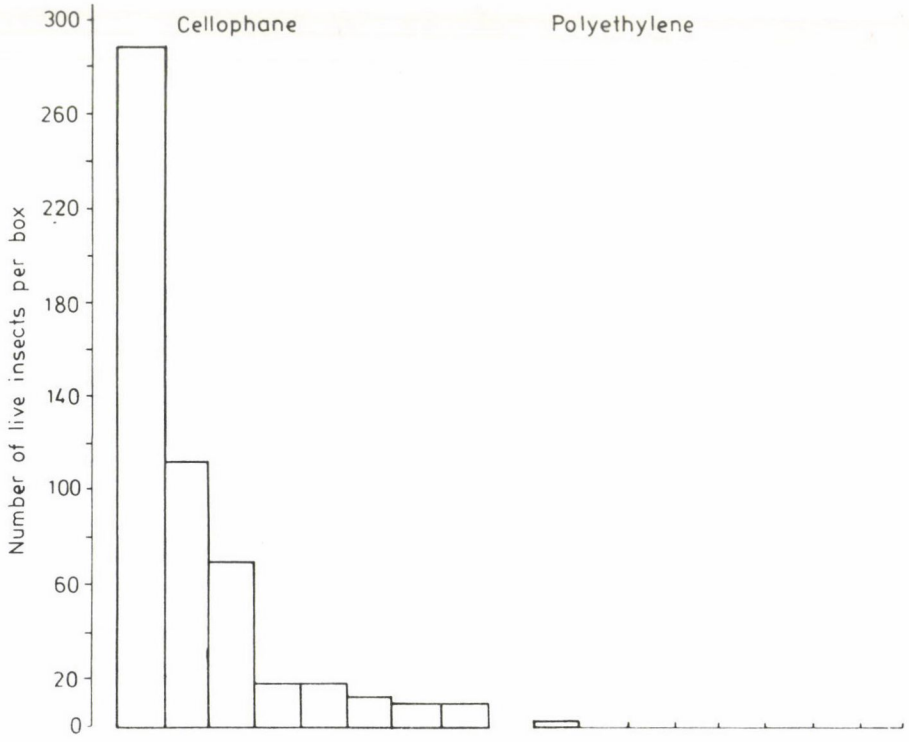


Fig. 2. Numbers of live insects per cellophane- or polyethylene-wrapped box 207 days after irradiation. Eight boxes were examined for each treatment

fested dates. Because of its heavy infestation, the storage of the fumigated lot was terminated after the examination on March 3, 1983.

The results of the examination of 8 lunch boxes after the first and the last periods of storage, i.e. 64 and 207 days after treatment, resp., are illustrated in Figs. 1 and 2.

Table 6

*Frequencies of numbers of live Oryzaephilus found in "lunch boxes" of artificially infested dates wrapped in cellophane or polyethylene films, treated with methyl bromide or gamma radiation and examined at five intervals of storage ranging from 64 to 207 days*

| Wrapping | Fumigation: Number of live insects per box | Storage time (day) 64 | 93 | 142 | 178 | 207 | Storage time effect | Irradiation: Number of live insects per box | Storage time (day) 64 | 93 | 142 | 178 | 207 | Storage time effect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cellophane | 0— < 20 | 1 | 0 | — | — | — | | 0— < 25 | 8 | 8 | 7 | 3 | 5 | |
| | 20— < 40 | 3 | 0 | — | — | — | | 25— < 50 | 0 | 0 | 1 | 3 | 0 | |
| | 40— < 60 | 3 | 1 | — | — | — | | 50— < 75 | 0 | 0 | 0 | 1 | 1 | |
| | 60— < 80 | 1 | 5 | — | — | — | | 75— <100 | 0 | 0 | 0 | 1 | 0 | |
| | 80— <100 | 0 | 1 | — | — | — | | 100— <125 | 0 | 0 | 0 | 0 | 1 | |
| | 100— <120 | 0 | 1 | — | — | — | ** | 125— <150 | 0 | 0 | 0 | 0 | 0 | ** |
| | 120— <140 | 0 | 0 | — | — | — | | 150— <175 | 0 | 0 | 0 | 0 | 0 | |
| | 140— <160 | 0 | 0 | — | — | — | | 175— <200 | 0 | 0 | 0 | 0 | 0 | |
| | 160— <180 | 0 | 0 | — | — | — | | 200— <225 | 0 | 0 | 0 | 0 | 0 | |
| | 180— <200 | 0 | 0 | — | — | — | | 225— <250 | 0 | 0 | 0 | 0 | 0 | |
| | 200— <220 | 0 | 0 | — | — | — | | 250— <275 | 0 | 0 | 0 | 0 | 0 | |
| | 220— <240 | 0 | 0 | — | — | — | | 275— <300 | 0 | 0 | 0 | 0 | 1 | |
| Polyethylene | 0— < 20 | 3 | 2 | — | — | — | | 0— < 25 | 8 | 8 | 8 | 8 | 8 | |
| | 20— < 40 | 5 | 0 | — | — | — | | 25— < 50 | 0 | 0 | 0 | 0 | 0 | |
| | 40— < 60 | 0 | 1 | — | — | — | | 50— < 75 | 0 | 0 | 0 | 0 | 0 | |
| | 60— < 80 | 0 | 1 | — | — | — | | 75— <100 | 0 | 0 | 0 | 0 | 0 | |
| | 80— <100 | 0 | 2 | — | — | — | | 100— <125 | 0 | 0 | 0 | 0 | 0 | |
| | 100— <120 | 0 | 1 | — | — | — | * | 125— <150 | 0 | 0 | 0 | 0 | 0 | Ø |
| | 120— <140 | 0 | 0 | — | — | — | | 150— <175 | 0 | 0 | 0 | 0 | 0 | |
| | 140— <160 | 0 | 0 | — | — | — | | 175— <200 | 0 | 0 | 0 | 0 | 0 | |
| | 160— <180 | 0 | 0 | — | — | — | | 200— <225 | 0 | 0 | 0 | 0 | 0 | |
| | 180— <200 | 0 | 0 | — | — | — | | 225— <250 | 0 | 0 | 0 | 0 | 0 | |
| | 200— <220 | 0 | 0 | — | — | — | | 250— <275 | 0 | 0 | 0 | 0 | 0 | |
| | 220— <240 | 0 | 1 | — | — | — | | 275— <300 | 0 | 0 | 0 | 0 | 0 | |

| Treatment or packaging effects | Storage time (day) 64 | 93 | 142 | 178 | 207 |
|---|---|---|---|---|---|
| Fumigation vs. irradiation in cellophane | *** | *** | — | — | — |
| Fumigation vs. irradiation in polyethylene | *** | *** | — | — | — |
| Cellophane vs. polyethylene after fumigation | ** | Ø | — | — | — |
| Cellophane vs. polyethylene after irradiation | Ø | Ø | Ø | *** | *** |

For further explanation and symbols see Table 2

Because the dominant infestation was due to *Oryzaephilus*, the statistical evaluation of the numbers of live insects was performed on the frequencies of occurrences of *Oryzaephilus* (Table 6).

Table 7 summarizes the number of insects escaped from the lunch boxes.

Table 7

*Numbers of insects found in the SCBs outside the "lunch boxes" of artificially infested dates*

| Date of inspection in 1983 | Days after disinfest. | Fumigated | | | | Irradiated | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Cellophane | | Polyethylene | | Cellophane | | Polyethylene | |
| | | Ephestia | Oryzaephilus | Ephestia | Oryzaephilus | Ephestia | Oryzaephilus | Ephestia | Oryzaephilus |
| February 2 | 64 | 2 | 178 | 0 | 66 | 1 | 0 | 0 | 0 |
| March 3 | 93 | 4 | 137 | 3 | 132 | 1 | 0 | 0 | 0 |
| April 21 | 142 | – | – | – | – | 0 | 0 | 0 | 2 |
| May 27 | 178 | – | – | – | – | 0 | 0 | 0 | 3 |
| June 25 | 207 | – | – | – | – | 0 | 2 | 0 | 0 |

—: not examined

The frequencies of percentage infestation and their statistical evaluation are given in Table 8.

The results clearly show that the disinfestation effect of gamma radiation is significantly superior to fumigation with methyl bromide.

The role of packaging material in preventing insect re-infestation is evident as the build-up of insect population in irradiated lots show a clear difference in favour of polyethylene wrapped packages. In almost all cases where irradiated polyethylene wrapped packages contained live insects, a close observation showed that the sealing was not airtight.

From the above mentioned results and discussion the following conclusions can be drawn:

— 0.7 kGy gamma radiation is more effective for disinfesting dates than commercial methyl bromide fumigation.

— Commercial cellophane wrapping is insufficient to prevent reinfestation.

— Properly sealed polyethylene wrapping is capable of preventing reinfestation under the storage conditions applied.

*

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Table 8

*Frequencies of percentage infectation of artificially infested dates packed in "lunch boxes" wrapped in cellophane or polyethylene films treated with methyl bromide or gamma radiation and examined at five intervals of storage ranging from 64 to 207 days*

| Wrapping | Percentage infested-fruits | Fumigation | | | | | Storage time effect | Irradiation | | | | | Storage time effect |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Storage time (day) | | | | | | Storage time (day) | | | | | |
| | | 64 | 93 | 142 | 178 | 207 | | 64 | 93 | 142 | 178 | 207 | |
| Cellophane | 5— <13 | 0 | 0 | — | — | — | Ø | 0 | 0 | 0 | 0 | 0 | ** |
| | 13— <21 | 0 | 0 | — | — | — | | 0 | 0 | 0 | 0 | 0 | |
| | 21— <29 | 0 | 0 | — | — | — | | 1 | 0 | 0 | 0 | 1 | |
| | 29— <37 | 0 | 0 | — | — | — | | 0 | 0 | 0 | 0 | 0 | |
| | 37— <45 | 0 | 0 | — | — | — | | 2 | 0 | 1 | 0 | 0 | |
| | 45— <53 | 0 | 0 | — | — | — | | 1 | 3 | 0 | 0 | 0 | |
| | 53— <61 | 0 | 0 | — | — | — | | 3 | 0 | 1 | 0 | 1 | |
| | 61— <69 | 0 | 0 | — | — | — | | 0 | 2 | 2 | 0 | 0 | |
| | 69— <77 | 0 | 0 | — | — | — | | 0 | 2 | 0 | 0 | 1 | |
| | 77— <85 | 1 | 0 | — | — | — | | 1 | 1 | 1 | 3 | 2 | |
| | 85— <93 | 2 | 4 | — | — | — | | 0 | 0 | 3 | 3 | 3 | |
| | 93— <101 | 5 | 4 | — | — | — | | 0 | 0 | 0 | 2 | 1 | |
| Polyethylene | 5— <13 | 0 | 0 | — | — | — | Ø | 0 | 1 | 0 | 0 | 0 | Ø |
| | 13— <21 | 0 | 0 | — | — | — | | 0 | 0 | 1 | 0 | 0 | |
| | 21— <29 | 0 | 0 | — | — | — | | 0 | 0 | 1 | 0 | 0 | |
| | 29— <37 | 0 | 0 | — | — | — | | 0 | 0 | 0 | 0 | 0 | |
| | 37— <45 | 0 | 0 | — | — | — | | 0 | 0 | 0 | 1 | 1 | |
| | 45— <53 | 1 | 0 | — | — | — | | 0 | 0 | 2 | 1 | 1 | |
| | 53— <61 | 0 | 0 | — | — | — | | 3 | 3 | 1 | 0 | 1 | |
| | 61— <69 | 1 | 0 | — | — | — | | 3 | 0 | 1 | 2 | 1 | |
| | 69— <77 | 2 | 2 | — | — | — | | 2 | 1 | 0 | 0 | 1 | |
| | 77— <85 | 2 | 2 | — | — | — | | 0 | 2 | 1 | 3 | 0 | |
| | 85— <93 | 2 | 2 | — | — | — | | 0 | 1 | 1 | 1 | 3 | |
| | 93— <101 | 0 | 2 | — | — | — | | 0 | 0 | 0 | 0 | 0 | |

| Treatment or packaging effects | Storage time (day) | | | | |
|---|---|---|---|---|---|
| | 64 | 93 | 142 | 178 | 207 |
| **Fumigation vs. irradiation in cellophane** | *** | *** | — | — | — |
| **Fumigation vs. irradiation in polyethylene** | ** | ** | — | — | — |
| **Cellophane vs. polyethylene, after fumigation** | *** | * | — | — | — |
| **Cellophane vs. polyethylene, after irradiation** | ** | Ø | * | *** | Ø |

**For further explanation and symbols see Table 2**

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# EMULSIFYING PROPERTIES OF *VICIA FABA* GLOBULINS

O. ANDERSSON, A. N. GUROV, N. V. GUROVA, H. SCHMANDKE and V. B. TOLSTOGUZOV

Central Institute of Nutrition, Academy of Sciences of the GDR, 1505 Potsdam-Rehbrücke, Arthur-Scheunert-Allee 114—116. GDR

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The emulsifying properties of the total globulin fraction and of the 11-S globulin of *Vicia faba* were studied in aqueous solution at different pH values. Since this study is only of model character decane was used as non-polar phase. The studied emulsifying properties are connected with the term emulsifying capacity which has been introduced into the literature. The problems in connection with it are discussed in detail.

**Keywords:** Emulsification, *Vicia faba*, globulins

The aim of the present study was to investigate the emulsifying properties of 11-S globulin of *Vicia faba* in dependence on the pH value. The work was restricted to the property which is in connection with the term emulsifying capacity (EC). This term has been used by different authors in modified forms which makes a comparison very difficult. In a paper of GUROV and co-workers (1983) the term emulsifying capacity is critically analyzed and the problems inherent in this term are made clear. In due consequence of this it was stated that the traditional term emulsifying capacity is only little suitable to be a standardized value to characterize the emulsifying properties. In the cited paper (GUROV et al., 1983) as a result of this opinion some proposals are made to the terminology, more suitable for the description of the emulsifying properties. The present paper is connected with these problems.

## 1. Material and methods

11-S *Vicia faba* globulin and the total globulin fraction of *Vicia faba* were used in aqueous solution. The globulin fraction is obtained from flour of the dehulled *Vicia faba* beans cultivar "Fribo" by extraction with NaCl. Previous to the extraction the flour is washed, isoelectrically at pH 4.8 to 5.0 for about 10 minutes. The extraction is carried out at first at pH 8.0 and then with 0.37 *M* NaCl at pH 5.0. After centrifugation the solution is diluted with water (1 : 1). The precipitate after dilution is *Vicia faba* globulin as a mesophase. The sedimentation analysis shows that the total globulin fraction contains



*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

about 50% 7-S globulin (vicilin) and about 50% 11-S globulin (legumin) (ANDERSSON et al., 1985).

From the solution of the total globulin fraction the 11-S globulin is prepared by heating from 20 °C to 80 °C at a pH of 7.0. By this the 7-S globulin is denatured, but not the 11-S globulin (SCHMANDKE et al., 1981). After cooling by dilution with water the precipitation is carried out at a pH of 5.0 and the 11-S globulin is extracted with 0.5 *M* NaCl, centrifuged, and reprecipitated by dilution. After the centrifugation the 11-S *Vicia faba* globulin is obtained as a mesophase containing about 95% 11-S and 5% 7-S globulin. For the measurement of the emulsifying action over a greater pH range the 11-S globulin is dissolved near the isoelectric point in 0.5 *M* NaCl, in other cases in 0.1 *M* NaCl. As non-polar phase decane ($C_{10}H_{22}$) was used because of its great purity and storage stability. The emulsification was realized in a cylindric vessel into which 7.0 $cm^3$ globulin solution were introduced. The solution is stirred by a blade agitator and decane is added by using a pump until the breakdown of the emulsion (conductivity measurement). The stirrer is rotated at 3500 r.p.m. and the addition rate for decane is 4.6 $cm^3\ min^{-1}$.

## 2. General considerations

In the literature (BARBER & WARTHESEN, 1982; MUSCHIOLIK & SCHMANDKE, 1982; SCHWENKE et al., 1981; SWIFT et al., 1961) the term emulsifying capacity (EC) means generally the ratio of the emulsified oil volume ($V_F$) before the breakdown of the solution to the mass of protein ($m_P$):

$$EC_a = \frac{V_F}{m_P} \tag{1}$$

The index $a$ (GUROV et al., 1983) is to indicate that it is an apparent emulsifying capacity. Frequently a part of the oil volume $V_{F,m_{P=0}} = V_{F,O}$ is emulsified in the absence of protein, then this part should be subtracted. This is illustrated in Fig. 1. The real oil volume emulsified by protein is

$$V_{F,P} = V_F - V_{F,O} \tag{2}$$

The values for the emulsifying capacity according to GUROV (1983) are valid for a constant mass concentration $\varrho_P$ of the protein in the aqueous phase $V_O$ (also called partial density):

$$\varrho_P = \frac{m_P}{V_O} \tag{3}$$

The unit of the emulsifying capacity in the international system is $(EC_a) = = m^3 kg^{-1}$, but in general $cm^3$ oil per 100 mg protein is used.

The restrictions in the usefulness of the definition for the emulsifying capacity according to equation (1) become clear if one considers the function for the maximum emulsified oil volume $V_F$ in dependence on mass concentration $\varrho_P$, which will be designated hereafter as emulsification function or as emulsification curve. Figure 1 is to explain that. Studies of the 11-S *Vicia faba* globulin led to emulsification curves, which may be ascribed to the two types A and B of the emulsification curves in Fig. 1. Neither of the types passes the origin of the coordinates. The emulsification curve *A* is linear for values below the saturation concentration $\varrho_{P,S}$. Then for the dependence of the emulsified oil volume $V_F$ on the protein mass $m_P$, the following equation is valid:

$$V_F = V_{F,O} + \frac{dV_{F,P}}{dm_P} m_P = V_{F,O} + EC_i m_P \tag{4}$$

From this it results

$$EC_a = \frac{V_F}{m_P} = \frac{V_{F,O} + V_{F,P}}{m_P} = \frac{V_{F,O}}{m_P} + EC_i \tag{5}$$

Equation (5) shows clearly (if $V_{F,O} \neq 0$) the hyperbolic change of the emulsifying capacity $(EC_a)$ according to equation (1) with changes of the

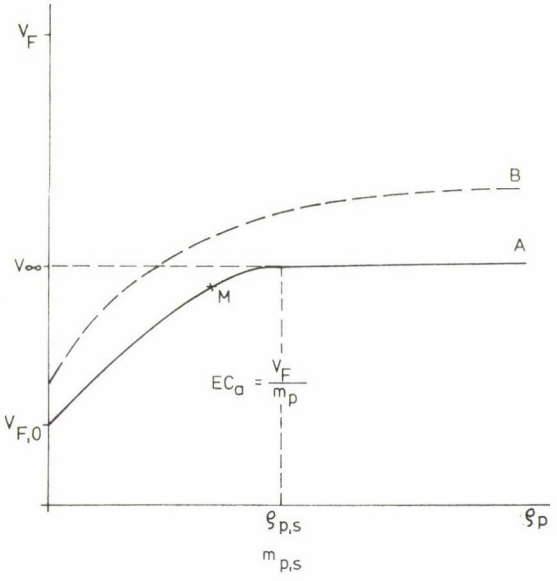


Fig. 1. Rough diagram of two types A and B of emulsifying functions for emulsified oil volume $(V_F)$ in dependence on the protein concentration $(\varrho_P)$ in the aqueous phase. Explanations in the text; $\varrho_P = \frac{m_P}{\varrho_O}$; $m_P = \varrho_P V_0$; $V_0$ = const.; M = point of measurement

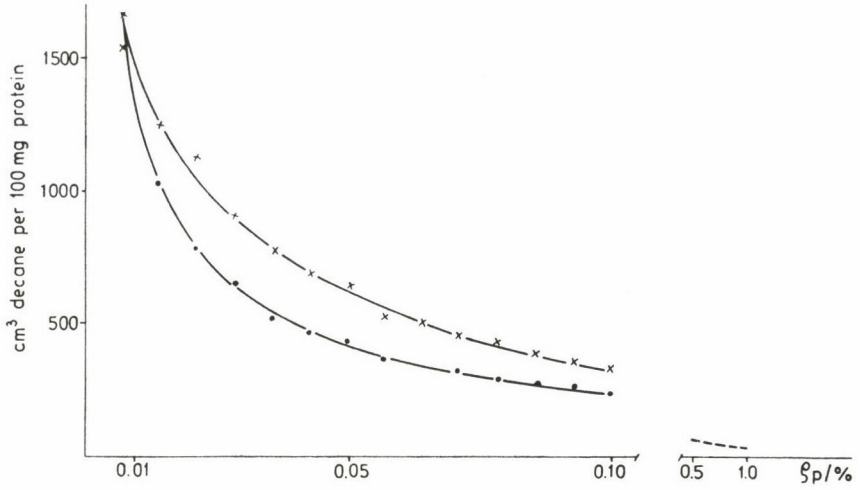


Fig. 2. Apparent emulsifying capacity ($EC_a$) as a function of the protein concentration ($\varrho_P$) according to the measured values of Fig. 3. Concentration of protein solution (0.1 *M* NaCl)/%; protein = 11-S globulin of *Vicia faba*; $-\times-$ = pH 7.0; $-\cdot-$ = pH 2.0

protein concentration. In Fig. 1 this is the change of the straight line between the measured point (M) and the origin of the coordinate system, when the measured point is shifted in the linear part of the emulsification curve *A*. It can easily be seen in Fig. 1 that changes of $EC_a$ are qualitatively equal in the nonlinear and in the saturation part. In Fig. 2 the quantitative change of $EC_a$ from measured emulsification curves is demonstrated as an example. This is to demonstrate the low aptness of $EC_a$ to be a reference standard. In equation (4) $EC_i$ is the tangential slope of the straight line. Generalizing, the tangent $dV_{F,P}$ per $dm_P$ can also be used in the non-linear part of the emulsification function for its characterization. For a constant volume ($V_O$) of the aqueous phase, with $m_P = \varrho_P V_O$

$$EC = \frac{dV_{F,P}}{dm_P} = \frac{1}{V_O} \frac{dV_{F,P}}{d\varrho_P} \tag{6}$$

can also be defined. The index i of $EC_i$ refers to intrinsic emulsifying capacity with

$$EC_i = \lim_{m_P \to 0} EC \tag{7}$$

As it has been stated by Gurov, a certain levelling of the influence of the different stirring speeds can be obtained when in equation (6) by division by $V_{F,O}$ a standardization to the emulsified oil volume is carried out for an absence of protein.

As a result of these considerations, GUROV and co-workers (1983) have proposed different terms for the validation of emulsifying properties:

— maximum oil volume of the emulsion in the saturation range $V_\infty = V_{F_{max}}$ (8)

— concentration at saturation: $\varrho_{P,S}$ (9)

— emulsifier activity: $A = \frac{dV_{F,P}}{dm_P}$ or $\frac{1}{V_{F,O}V_O} \cdot \frac{dV_{F,P}}{d\varrho_P}$ (10)

— intrinsic activity of the emulsifier: $A_i = \lim_{m_P \to 0} A$ (11)

## 3. Results and discussion

The maximum volumes $V_F$ of emulsified decane as a function of the protein concentration $\varrho_P$ of the studied protein solutions are shown in Figs. 3 to 5. It is striking that in Figs. 4 and 5 the emulsification curves can be described very well by two lines according to the model curve *A* in Fig. 1. The emulsification curves in Fig. 3 correspond to the model curve *B* of Fig. 1. It is remarkable for most of the emulsification curves that by extrapolation in the lower concentration range, the curves did not lead to the measured value $V_{F,O}$. This fact cannot yet be explained.

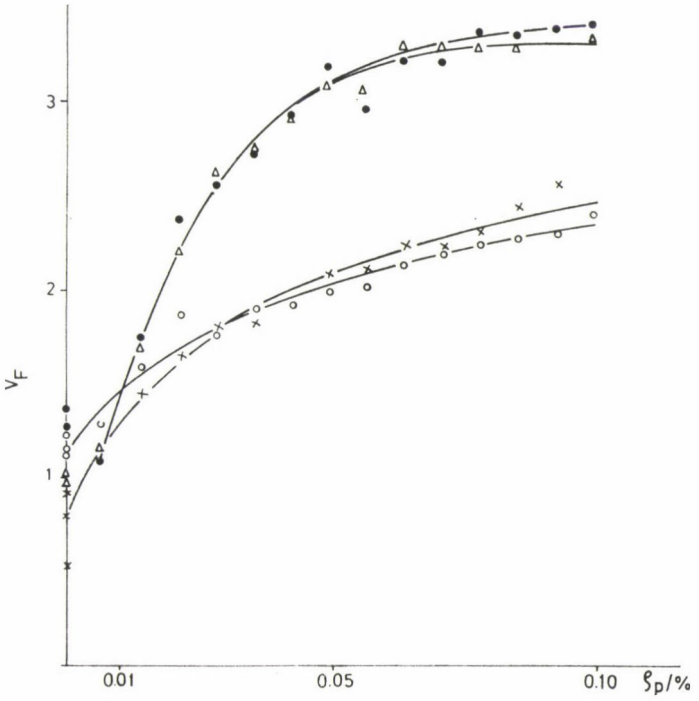


Fig. 3. Emulsified volume of decane $V_F$, referred to 1 cm³ protein solution, as a function of the protein concentration $\varrho_P$ (11-S *Vicia faba* globulin; 0.1 *M* NaCl) at different pH: —•— = pH 7.0; —△— = pH 8.0; —×— = pH 2.0; —○— = pH 3.0

Table 1

*Parameters of the measured emulsifying functions*

| Emulsifier | $V_{F,0}$ cm³ measured | $V_{F,0}$ cm³ extrapol. | $V_\infty$ cm³ | $\varrho_{P,S}$ g per 100 cm³ | $A_i$ cm³ decane per mg protein | $\sigma_{F,0}$ % | $\sigma_{F,0}$ % | $\varrho_k$ g per 100 cm³ | n |
|---|---|---|---|---|---|---|---|---|---|
| 11-S *V.f.* 0.1 *M* NaCl pH 2 | 0.93 | 0.93 | — | — | 3.4 | 49 | 74 | 0.020 | 1.28 |
| 11-S *V.f.* 0.1 *M* NaCl pH 3 | 1.17 | 1.17 | — | — | 2.8 | 54 | 73 | 0.027 | 1.18 |
| 11-S *V.f.* 0.1 *M* NaCl pH 7 | 1.33 | 0.50 | 3.40 | ~0.08 | 7.5 | 35 | 78 | 0.008 | 1.60 |
| 11-S *V.f.* 0.1 *M* NaCl pH 8 | 1.01 | 0.50 | 3.36 | ~0.08 | 7.5 | 35 | 77.5 | 0.008 | 1.73 |
| 11-S *V.f.* 0.5 *M* NaCl pH 5 | 1.14 | 0.86 | 3.17 | 0.04 | 6.1 | 46 | 76.5 | 0.013 | 2.89 |
| 11-S *V.f.* 0.5 *M* NaCl pH 7 | 1.20 | 0.70 | 2.64 | 0.04 | 5.2 | 44 | 73 | 0.017 | 3.64 |
| *V.f.* globulin 0.1 *M* NaCl pH 7 | 0.95 | 0.61 | 3.36 | 0.06 | 6.0 | 38 | 77.5 | 0.011 | 2.16 |

The characterization of the emulsification curves of Figs 4 and 5 with the parameters (2; 8; 9; 11) is without any problem. The results are summarized in Table 1. The determination of these parameters also for the emulsification curves in Fig. 3 is connected with a considerable mistake. Here, it is to be considered, whether these data are intended for ranges of practical application with little demand for accuracy or whether a high accuracy is

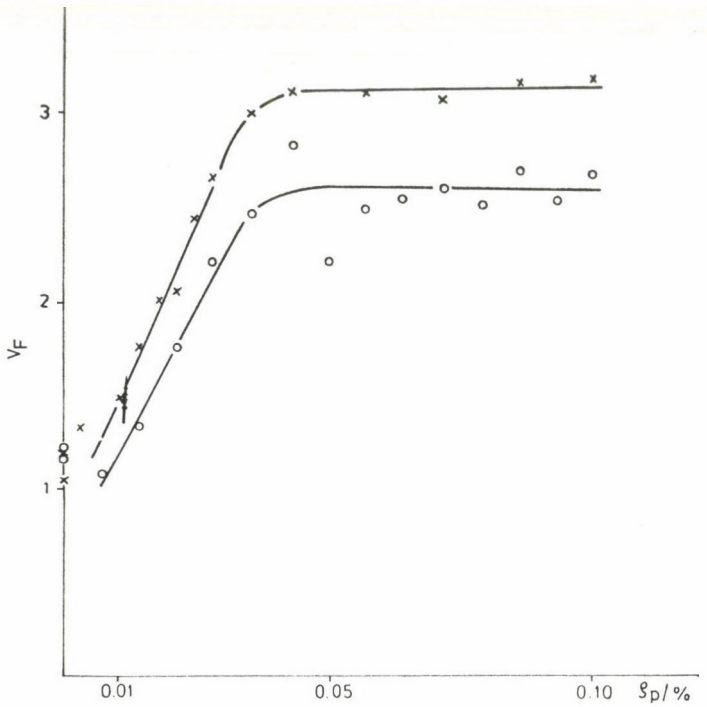


Fig. 4. Emulsified volume of decane $V_F$, referred to 1 cm³ protein solution, as a function of the protein concentration $\varrho_P$ (11-S *Vicia faba* globulin; 0.5 *M* NaCl) at different pH: $-\times-$ = pH 5.0; $-\circ-$ = pH 7.0

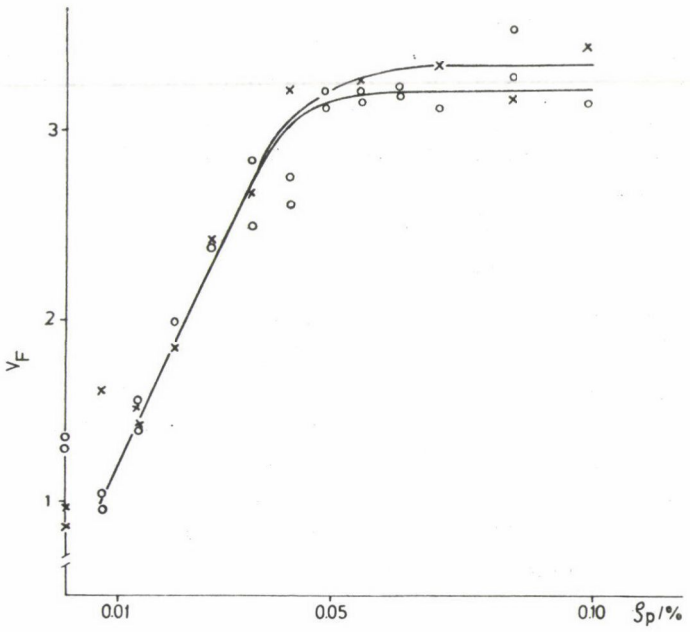


Fig. 5. Emulsified volume of decane $V_F$, referred to 1 cm³ protein solution, as a function of $\varrho_P$ at a pH of 7 for two protein solutions (total globulin fraction of *Vicia faba*) prepared at different times

required as for instance in fundamental research with subsequent molecular interpretations.

In the latter case it is suitable to describe the emulsification curves by an analytical expression the parameters of which admit certain physical interpretations and which can characterize the emulsification curve in the frame of the concentration of information processing. The following equation may be proposed:

$$V_F = V_{F,O} + \frac{\varrho^n}{\varrho^n + k}(V_\infty - V_{F,O}) \qquad (12a)$$

wherein $k = \varrho_k^n \cdot \varrho_k$ indicates a critical concentration. At first view, this equation seems to be of a particular advantage for measured curves of the *A* type in Fig. 1. With $n = 1$ and $\ll k$ (12a) it may be written, below saturation, in the linear form

$$V_F = V_{F,O} + \frac{\varrho}{k}(V_\infty - V_{F,O})$$

With $n = 1$ and $k \ll \varrho$ one gets $V_F = V_\infty$, thus describing the saturation range.

However, the application of this equation to the obtained curves showed a too great error since the differences between k and $\varrho$ are too small and

with that the conditions of estimation are not sufficiently fulfilled. The adaptation of equation (12a) is better when $1 < n$ and the emulsification curves correspond more to type *B* in Fig. 1. Then an adaptation can be reached almost until the drawing accuracy (cf. Fig. 7). The parameters k and n can easily be obtained when the equation (12a) becomes a straight line by a double logarithmic graph:

$$\log \frac{V_\infty - V_F}{V_F - V_{F,O}} = \log k - n \log \varrho \qquad (13a)$$

By varying $V_\infty$ the best conformity with a straight line can be found with the measured values. From that, a clear recipe results for the fixation of $V_\infty$ if this value could not be measured for given reasons. The linear part of the curve *A* in Fig. 1. below saturation changes towards *B* when another graphic description of the emulsification curves is chosen. From the practical point of view the demand is to figure the emulsification curves in such a way that one can immediately read off how much oil ($V_F$) is emulsified in relation to the total volume ($V_F + V_O$). This ratio is the volume concentration

$$\varrho_F = \frac{V_F}{V_F + V_O} = f(\varrho_P) \qquad (14)$$

which is to be regarded as a function of the protein concentration in the aqueous phase.

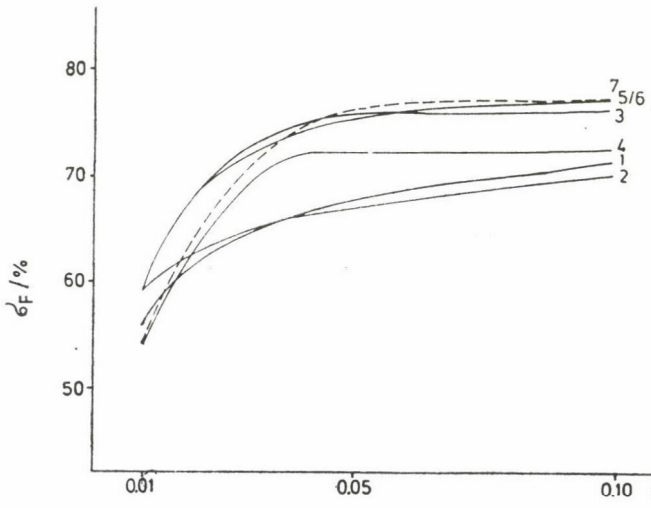


Fig. 6. The measured emulsifying function as volume concentration ($\sigma_F$) of the emulsified decane in dependence on the protein concentration ($\varrho_P$). 1: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 2; 2: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 3; 3: 11-S *Vicia faba*, 0.5 *M* NaCl, pH 5; 4: 11-S *Vicia faba*, 0.5 *M* NaCl, pH 7; 5: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 7; 6: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 8; 7: total globulin of *Vicia faba*, 0.1 *M* NaCl, pH 7

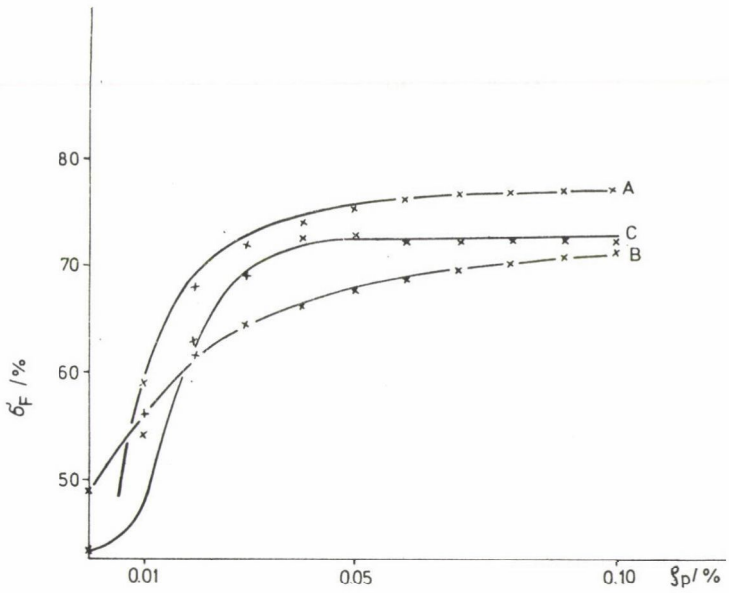


Fig. 7. Demonstration of the adaptation of equation 12b. $\times\times\times$ = measured points; ——— = curve calculated according to the equation;

A: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 7, $\sigma_F = 35 + \frac{\varrho_P^{1.6} \cdot 43}{\varrho_P^{1.6} + 0.083^{1.6}}$

B: 11-S *Vicia faba*, 0.1 *M* NaCl, pH 2, $\sigma_F = 49 + \frac{\varrho_P^{1.28} \cdot 25}{\varrho_P^{1.28} + 0.020^{1.28}}$

C: 11-S *Vicia faba*, 0.5 *M* NaCl, pH 7, $\sigma_F = 44 + \frac{\varrho_P^{3.64} \cdot 29}{\varrho_P^{3.64} + 0.017^{3.64}}$

Corresponding to equations (12a) and (13a) it follows

$$\sigma_F = \sigma_{F,O} + \frac{\varrho^n}{\varrho^n + k} (\sigma_{F,\infty} - \sigma_{F,O}) \tag{12b}$$

and

$$\log \frac{\sigma_{F,\infty} - \sigma_F}{\sigma_F - \sigma_{F,O}} = \log k - n \log \varrho \tag{13b}$$

Figure 6 shows the volume concentration of the emulsified decane as a function of the concentration of protein in the studied protein solutions. It can be read directly that, under the experimental conditions, the portion of decane in the emulsion can amount up to 60 to 80% at most. The exactness of the adaptation of equation (12b) to the emulsification curves of Fig. 6 is demonstrated in Fig. 7 in order to obtain a better clearness only for three

extreme emulsification curves. The corresponding parameters according to equation (12b) are given in Table 1 for all emulsification curves.

The comparison of the parameters of the two chosen graphs of the emulsification curves $V_F = f(\varrho_P)$ and $\sigma_F = f(\varrho_P)$, respectively, is possible with the values given in Table 1. The parameters in columns 1 to 4 can be very exactly detected for some emulsification curves type *A* in Fig. 1, but for some other measured emulsification curves they can not or only approximately be determined. In comparison with $\varrho_k$ the better descriptiveness is an interesting advantage of the saturation concentration ($\varrho_{P,,S}$).

A disadvantage is to be seen in the fact that $\varrho_{PS}$ could not be unambiguously fixed for all emulsification curves. An advantage of $A_i$ can be seen in the leaning against the proper content of the assertion of the emulsifying capacity, if, essentially, the emulsification curves are characterized with it up to the saturation concentration. The $A_i$ is less representative for emulsification curves of type *B*. In this case the characterization by the parameters of columns 5 to 8 according to equation (12b) is substantially more advantageous. This advantage is by the representation of the emulsifying function by $\sigma_F = f(\varrho_P)$ because in this way type A turns to type B. The maximum values $\sigma_{F,\infty}$ and the other parameters are determined according to an unambiguous instruction. With these parameters the measured emulsifying function can be constructed more exactly. As to the dependence of the maximum volume concentration $\sigma_{F,\infty}$ of the emulsified decane on the pH of the protein solution, it can be said that the maximum value of about 78% of emulsified decane in the emulsion is reached with 11-S *Vicia faba* globulin and 0.1 *M* NaCl at pH 7 and 8. Under the same conditions, but at pH 2 and 3, the maximum value is 74%. With 0.5 *M* NaCl a reciprocal dependence on the pH seems to occur. With 0.1 *M* NaCl at a pH of 7 there is no difference between the total globulin and the 11-S *Vicia faba* globulin. Altogether, the differences are only small and as follows from the values 73 to 78%, they lie close together. For some emulsifying functions the saturation range $\varrho_P \geqq \varrho_{P,S}$ begins at about 0.04% and it depends on the fact at which percentage of $\sigma_{F,\infty}$ (e.g. 95 or 99%) $\varrho_{P,S}$ is determined. This settled, the saturation concentration can unambiguously be calculated according to equation (12b).

## *Nomenclature*

| | |
|---|---|
| EC | emulsifying capacity |
| $EC_a$ | apparent emulsifying capacity |
| $EC_i$ | intrinsic emulsifying capacity |
| A | activity of emulsifier |
| $A_i$ | intrinsic activity of emulsifier |
| $m_P$ | mass of protein |

| | |
|---|---|
| $V_O$ | volume of aqueous phase |
| $V_F$ | maximum emulsified oil volume (up to emulsion collapse) |
| $V_{F,O}$ | emulsified oil volume when protein is absent |
| $V_{F,P} = V_F - V_{F,O}$ | oil volume emulsified by action of protein |
| $V_\infty = V_{F_{max}}$ | maximum oil volume of the emulsion in the saturation range |
| $\sigma_F = \frac{V_F}{V_{ges}} = \frac{V_F}{V_F + V_O}$ | volume concentration of the emulsified oil |
| $\sigma_{F,O} - \sigma_F$ | when protein is absent |
| $\sigma_{F,\infty} - \sigma_F$ | in the saturation range |
| $\varrho_P = \frac{m_P}{V_O}$ | mass concentration or partial density of protein |
| $\varrho_{P,S}$ | saturation mass concentration |
| $\varrho_K$ | critical mass concentration |

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# ENZYME ACTIVITIES OF FINNISH WHEATS AND FLOURS

A. PÁRKÁNY-GYÁRFÁS[a], L. VÁMOS-VIGYÁZÓ[a], P. KOIVISTOINEN[b] and H. SALOVAARA[b]

[a] Central Food Research Institute, H-1022 Budapest, Herman Ottó út 15. Hungary
[b] EKT Department of Food Chemistry and Technology, University of Helsinki, Faculty of Agriculture and Forestry, SF-00710, Helsinki 71. Finland

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The activities of some technologically important enzymes were determined in three wheat samples grown in Finland, and in the respective flours. Amylase activity of wheat varied over two orders of magnitude and was, in one of the samples (A) more than 4-fold the upper limit of the range found in Hungarian wheats. Milling (to a flour yield of 62–63%) reduced amylase activity by 59–66%. Peptide hydrolase activity in wheat was inversely related to amylase activity, without showing extreme values. Milling losses amounted to 23–47%. Lipoxygenase activities were similar in the three wheat samples and within the range found in Hungarian wheats. Milling losses were highest for this enzyme (67–74%). Peroxidase activities in the three wheat samples were also similar and somewhat below the values found in Hungarian wheats. Milling losses ranged from 34 to 56%. The reduction of the different enzyme activities by the milling process was consistent with data published on the locations of the individual enzymes in the wheat kernel. Excessive amylase activity in sample A manifested itself in the baking properties.

**Keywords:** Wheat, flour, enzyme activity, baking test

The activities of endogenous enzymes may play an important role in breadmaking (FOX & MULVIHILL, 1982). As the localization of the individual enzymes in the wheat kernel is different, their activities in flour will be highly dependent on milling conditions. Factors influencing the levels of enzyme activities in wheat are cultivar, location and climate. Cool and rainy climate might lead to increased amylase activities both in wheat and in flour. This again might result in poor bread quality (sticky crumb, lower volume). Wheats of elevated amylase activity are relatively frequent in Finland. As not much is known about the enzymes in Finnish wheats it seemed expedient to determine the activities of several technologically important enzymes in wheat and flour samples produced in Finland. Methods applied earlier for similar purposes in Hungary were used throughout the study to permit a comparison of results.

The present study deals with the determination of amylase, peptide hydrolase, peroxidase and lipoxygenase activities of three wheat samples grown in Finland, and of the respective flours. Baking trials were performed as well.

*Akadémiai Kiadó, Budapest*
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## 1. Materials and methods

### *1.1. The wheats and flours*

The wheat samples A, B and C all belonged to the spring cultivar Tähti and were grown in Finland, 1980. Some data of routine analysis of the wheats and the respective flours as carried out at the Department of Food Chemistry and Technology (EKT) of the University of Helsinki are given in Table 1.

Table 1

*Some routine analysis data of three samples of the spring wheat Tähti grown in Finland in 1980*

| | Sample | | |
|---|---|---|---|
| | A | B | C |
| *Grain* | | | |
| Moisture content (%)[a] | 12.80 | 12.50 | 12.07 |
| Falling number (7 g) | 62 | 113 | 288 |
| Germination capacity (Vitascope, %) | 30 | 68 | 98 |
| Hectolitre weight (kg) | 74.5 | 75.1 | 77.3 |
| Pelshenke test (min) | 93 | 103 | 105 |
| Zeleny-number | 35.3 | 46.5 | 32.5 |
| *Flour* yield (%) | 63.3 | 62.7 | 62.1 |
| Moisture content (%)[a] | 13.99 | 13.67 | 14.15 |
| Farinogram (AACC 54-21) | | | |
| — absorption (%) | 65.4 | 67.3 | 68.4 |
| — arrival time (min) | 2 | 2 | 3.5 |
| — dough development time (min) | 3 | 3.5 | 5.5 |
| — stability (min) | 4.5 | 7 | 8 |
| — drop off (B.U. per 20 min) | 140 | 120 | 80 |
| Baking test (modified Rapid-Mix-Test) | | | |
| — Volume (100 g flour) | 510 | 560 | 600 |

[a] Determined by drying to constant mass at 105 °C, simultaneously with enzyme activity measurements.

### *1.2. Preparation of enzyme extracts*

The wheat samples were ground in a grinder (type Savaria, Keripar, Szombathely) to a particle size of maximum 800 $\mu$m. Ten to fifty g of the wheat grits and the flour, respectively, were shaken with 100 $cm^3$ distilled water for 60 min at 5 °C on a table shaker and subsequently centrifuged at 4 °C and 20 000 r.p.m. for 20 min (Model J 2-21 centrifuge, rotor: J 20, Beckman, Fullerton, California). The filtered supernatant was used for measurements.

*1.3. Methods of determining enzyme activities*

The Phadebas Amylase Test (Pharmacia AB, Uppsala, Sweden) was used for amylase activity determination (PÁRKÁNY-GYÁRFÁS & VÁMOS-VIGYÁZÓ, 1979).

Peptide hydrolase (BAPA-ase) activity was assayed as described by KRUGER (1971).

Peroxidase activity determinations were carried out using o-phenylene diamine as hydrogen donor (TEMESVÁRI et al., 1981), while lipoxygenase activity was measured by the UV-method (PÁLOSI-SZÁNTHÓ et al., 1981).

*1.4. Calculation of enzyme activities*

All enzyme activities were determined from 2 parallel measurements each, carried out with three enzyme-containing extracts. Enzyme activities were calculated from the linear sections of the regression curves obtained when plotting absorbance vs. reaction time. The slope of the regression curve was taken as enzyme activity and expressed as $\Delta A$ per min. A change in absorbance of $10^{-3}$ $\Delta A$ per min was considered unit enzyme activity and this was related to 1 g of solids content. Results were evaluated by analysis of variance (carried out in cases of inhomogeneous variances after logarithmic transformation). The comparison of the mean values of the activities in the samples was based on the least significant difference at the probability level of 95%.

## 2. Results

*2.1. Enzyme activities*

The enzyme activity values as related to solids contents of the wheat and flour samples are given in Tables 2 to 5.

*2.2.1. Amylase activity.* From the three wheat and flour samples, amylase activities of wheat A and of the flour prepared of it were considerably higher than those of the other two wheat and flour samples.

Table 2

*Amylase activity*

| Sample | A | | B | | C | |
|---|---|---|---|---|---|---|
| Activity | U $g^{-1}$ | % | U $g^{-1}$ | % | U $g^{-1}$ | % |
| Wheat | 2200 ± 75 | 100 | 431 ± 1 | 20 | 48 ± 2 | 2 |
| Flour | 746 ± 14 | 100 | 178 ± 6 | 24 | 17 ± 0.4 | 2 |
| Ratio of activities flour : wheat | 0.34 | | 0.41 | | 0.35 | |

U = activity unit = $10^{-3}$ $\Delta A$ $min^{-1}$ (A = absorbance)

Table 3

*Peptide hydrolase activity*

| Sample | A | | B | | C | |
|---|---|---|---|---|---|---|
| Activity | U g$^{-1}$ | % | U g$^{-1}$ | % | U g$^{-1}$ | % |
| Wheat | 365 ± 7 | 73 | 469 ± 11 | 93 | 503 ± 9 | 100 |
| Flour | 212 ± 2.3 | 59 | 359 ± 3.5 | 100 | 267 ± 2.7 | 74 |
| Ratio of activities flour: wheat | 0.58 | | 0.77 | | 0.53 | |
| Ratio of amylase to peptide hydrolase wheat | 6.2 | | 0.9 | | 0.1 | |
| Ratio of amylase to peptide hydrolase flour | 3.5 | | 0.5 | | 0.06 | |

Symbols as in Table 2

Table 4

*Peroxidase activity*

| Sample | A | | B | | C | |
|---|---|---|---|---|---|---|
| Activity | U g$^{-1}$ | % | U g$^{-1}$ | % | U g$^{-1}$ | % |
| Wheat | 24 340 ± 480 | 93 | 26 250 ± 450 | 100 | 25 180 ± 470 | 96 |
| Flour | 13 990 ± 170 | 84 | 11 500 ± 155 | 69 | 16 690 ± 230 | 100 |
| Ratio of activities flour: wheat | 0.57 | | 0.44 | | 0.66 | |

Symbols as in Table 2

Table 5

*Lipoxygenase activity*

| Sample | A | | B | | C | |
|---|---|---|---|---|---|---|
| Activity | U g$^{-1}$ | % | U g$^{-1}$ | % | U g$^{-1}$ | % |
| Wheat | 25 500 ± 520 | 98 | 23 100 ± 380 | 89 | 26 000 ± 260 | 100 |
| Flour | 6 700 ± 80 | 79 | 6 700 ± 100 | 79 | 8 500 ± 90 | 100 |
| Ratio of activities flour: wheat | 0.26 | | 0.29 | | 0.33 | |
| Ratio of peroxidase to lipoxygenase wheat | 0.98 | | 1.14 | | 0.97 | |
| Ratio of peroxidase to lipoxygenase flour | 2.09 | | 1.72 | | 1.96 | |

Symbols as in Table 2

The amylase activities of wheat sample and flour sample B were about 1/5 and 1/4, respectively, of those of the A samples. Activity values of wheat and flour C were the lowest: they amounted to but 2% of the values found in sample A (Table 2).

*2.2.2. Peptide hydrolase activity.* The order of the peptide hydrolase activities in the three wheat samples was reversed as compared to the amylase

activities (Table 3). From the flours, peptide hydrolase activity was highest in sample B and lowest in sample A. The peptide hydrolase activity of flour B was nearly identical with that of wheat A. Differences in peptide hydrolase activities between both the wheat and the flour samples, were significant.

*2.2.3. Peroxidase activity.* Peroxidase activity (Table 4) was highest in wheat B and lowest in wheat A. From the flour samples, C had the highest and B the lowest peroxidase activity. The differences between the peroxidase activities of both wheats and flours proved to be significant.

*2.2.4. Lipoxygenase activity.* Lipoxygenase activity was highest in wheat C and lowest in wheat B. The differences between the lipoxygenase activities of all three wheat samples were significant (Table 5). From the flour samples, A and B had identical lipoxygenase activities. The activity of flour C was considerably higher (by 27%).

## 3. Conclusions

### *3.1. Ratios of the different enzyme activities*

In flour processing the ratio of amylolytic to proteolytic enzymes is quality determining. The differences between the three wheats and flours were most pronounced in these activities. Of course, peptide hydrolase represents only one group of the proteolytic enzymes in wheat (FOX & MULVIHILL, 1982). In the three wheat samples amylase and peptide hydrolase activities were inversely related. With wheats grown in Hungary no such relationship was ever observed; amylase and peroxidase activities seemed to be inversely related in samples of different Hungarian cultivars from different years and locations (PÁRKÁNY-GYÁRFÁS, 1982). Amylase activity was found to be equally highest in wheat A and flour A (Table 2), the ratio of amylase – peptide hydrolase was 6.2 and 3.5, respectively (Table 3). In samples B the respective ratios were 0.9 and 0.5, in samples C they were 0.1 and 0.06. The ratios in the flours were directly related to the ratios in the wheats. This shows the ratio of the two enzymes to be practically unaffected by milling and its value found in flour to be determined by that of wheat.

The ratios of the peroxidase and lipoxygenase activities were around 1.0 in wheats and around 2 in flours (Table 5). This shows lipoxygenase activity to be much more reduced in the milling process than peroxidase values.

### *3.2. Comparison of enzyme activities in flours and wheats*

The results tabulated show that, as compared to the values found in wheat, lipoxygenase and amylase activities were much lower in flour (29% and 37% resp., on the average) while peroxidase and peptide hydrolase activities were less reduced (56% and 63%, resp., on the average).

Peptide hydrolase activity is reported to be concentrated in the endosperm, i.e. the inner part of the kernel (EVERS & REDMAN, 1973; KRUGER, 1973). This supports our finding concerning the ratio of this enzyme in wheat and flour. Lipoxygenase was found to be concentrated in the scutellum and embryo, while activity was low in the endosperm (BLAIN & TODD, 1955). This explains the drastic reduction in its activity on milling. The poor heat stability (IRVINE, 1959) of this enzyme might also have contributed to the observed decrease in activity.

The relatively high value of the flour : wheat activity ratio suggests peroxidase to be located mainly in the inner parts of the wheat kernel. The pertinent data of the literature are contradictory, according to HONOLD and STAHMANN (1968) the enzyme is concentrated in the pericarp. This finding is borne out for immature wheat by KRUGER and LABERGE (1974). According to the latter authors, maturation brings about the accumulation of peroxidase in the endosperm.

The loss of nearly two thirds of the amylase activity upon milling is consistent with the findings of BANKS and co-workers (1972) and of KRUGER (1972) according to which $\alpha$-amylases of wheat are mainly located in the pericarp. Although the method used for activity measurements in the present study was not specific for $\alpha$-amylases, a possible interference of $\beta$-amylases might be but slight as these are reported to be present in ungerminated wheat to about 80% in a latent form (ROWSELL & GOAD, 1962).

### *3.3. Relationships between enzyme activities and technological data*

Comparing the data of Table 1 to the enzyme activities measured in the wheats and flours (Tables 2–5), the following relationships could be established.

As expected, the falling number (FN) was inversely related to amylase activity of wheat (MATHEWSON & POMERANZ, 1978). Of course, all the grain tests varying in the same sense as the falling number were also found to be inversely related to amylase activity. Dough development time and stability as well as bread volume were inversely while the drop off time was directly related to amylase activity of flour.

### *3.4. Comparison of enzyme activities in Finnish and Hungarian wheats*

A comparison of the data obtained for the Finnish wheat samples with data measured in samples grown in Hungary (PÁRKÁNY-GYÁRFÁS, 1982) permits to draw conclusions as to the variability of the activity levels of the enzymes studied with cultivar and/or climate.

Amylase activities in a great number of Hungarian wheat samples of different cultivars, years and locations were found to be in the range of the

Finnish samples B and C. No sample of amylase activity above 500 U $g^{-1}$ has been found in Hungary so far. Peptide hydrolase activities were very similar in the wheats analyzed from the two countries. Peroxidase activities in Hungarian wheat samples were in the range of 38–58 kU $g^{-1}$, while lipoxygenase activities ranged from 8.5 to 57.0 kU $g^{-1}$. The values in the Finnish samples were somewhat lower for peroxidase activity, and within the range of the values in Hungarian wheats for lipoxygenase activity.

The Hungarian wheats of amylase activities below 500 U $g^{-1}$ proved satisfactory in breadmaking. This was confirmed also for Finnish wheats by the baking tests (Table 1), while sample A of extremely elevated amylase activity gave, among others, a loaf of considerably smaller volume. The higher peroxidase activities of the Hungarian wheats may be considered advantageous in breadmaking as this enzyme is believed to have a role in protein polymerization, thus improving the rheological properties of dough (Fox & Mulvihill, 1982).

In order to study the influence of the climate on the activities of the enzymes studied, in particular of amylase, it was decided to grow, on experimental parcels in Hungary, the given Finnish cultivar along with a cultivar approved in the former country and follow the changes in activities from flowering till harvest. The results of this work will be reported in a separate paper.

*

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# BOOK REVIEW

**Food chemistry**

O. R. FENNEMA (Ed.)

Marcel Dekker, New York, 1985; 1008 pages. Second (revised and expanded) edition

The book is the Volume 15 of Food Science and Technology, a series of monographs and textbooks. It contains 16 chapters, subject- and chemical indices. At the end of the chapters the list of literature sources consists of two parts, the references and general bibliography promoting the research work. The text is well illustrated with tables and figures facilitating quick comprehension and understanding of essential materials.

The structure of the book is quite clear. The text covers in sequence the major and minor constituents of foods, the characteristics of edible fluids of animal origin, and of edible plant tissues, and in the last part the problems of shelf life of foods. The work excellently covers all aspects of food chemistry.

The book was written by internationally recognized food chemists, almost every chapter is written by different authors. The book is suitable for a two-semester course on food chemistry; several chapters are useful as primary source materials for special courses of graduate level but it also gives a solid background for researchers in the field of food science and chemistry including nutrition, biology and biochemistry.

I. VARSÁNYI

*Akadémiai Kiadó, Budapest*
*D. Reidel, Dordrecht*

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